Université de Toulouse

THÈSE

En vue de l'obtention du

DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE

Délivré par :
Institut National Polytechnique de Toulouse (INP Toulouse)

Discipline ou spécialité :

Développement des plantes

Présentée et soutenue par :
Cristina Ioana BARSAN

le : mercredi 10 novembre 2010

Titre :

CARACTERISATION DU CHROMOPLASTE DE TOMATE PAR APPROCHE PROTEOMIQUE

JURY

POPESCU Octavian, IB Bucarest - Président du jury
BREZEANU Aurelia, IB Bucarest
LATCHE Alain, INP-ENSA Toulouse
ROTHAN Christophe, INRA Bordeaux
MIRANCEA Nicolae, IB Bucarest
PECH Jean-Claude, INP-ENSA Toulouse
VASSU Tatiana, Faculté de Biologie Bucarest

Ecole doctorale :
Sciences Ecologiques, Vétérinaires, Agronomiques et Bioingénieries (SEVAB)

Unité de recherche :
Génomique et Biotechnologie des Fruits INRA-INP/ENSAT, Institut de Biologie (IB) Bucarest

Directeur(s) de Thèse :
PECH Jean-Claude
BREZEANU Aurelia

Rapporteurs :
ROTHAN Christophe, INRA Bordeaux
MIRANCEA Nicolae, IB Bucarest

CONTENT

CHAPTER IV

Aknowledgments

I would like to thank Dr Mondher Bouzayen and Dr. Madalin Enache for gracefully allowing me to be part of their teams. To my two supervisors prof. Dr. Jean –Claude Pech and Dr. Aurelia Brezeanu for their guidance, advice, involvement and support in my work. I would have never made it if without the help of Dr. Alain Latche who was there in all the phases of the project. A big thank you to Dr. Paloma Sanchez-Bel, Dr.Isabel Egea, Dr. Mohammed Zouine, Dr. Cezar Rombaldi, Dr. Eduardo Purgatto Dr. Marcel Kuntz and Isabelle Mila for their important contribution to the project and for sharing their knowledge with me.

This project was done in tight collaboration with the IPBS – IFR40 unit, thank you all, especially Michel Rossignol, Gisele Borderies and Carole Pichereaux.

I would like to thank to all the GBF team, for their support, advice and friendship, you all contributed to the accomplishment of this thesis.

All the microscopy was done ant the Imagery platforme under the advice of Alain Jauneau – thank you.

Thank you Dr. Cristian Banciu and Dr. Medana Zamfir who brought me support in all the administrative jungle. And I would like to thank to all my colleagues from the Institute of Biology Bucharest for their friendship and support.

My scholarship was granted by the French Embassy in Bucharest and the Crous who contributed also to the aquision of a brand new PC. Thank you for making my staying in France possible.

And last but not the least I would like to thank to my family for bearing with me all these years and for being there for me all the way.

PUBLICATIONS

Several papers have been published during the preparation of the present thesis and are included in chapter I, Chapter II and Chapter III respectively:

Barsan C., Egea I., Bian W., Purgatto E., Latché A., Chervin C., Bouzayen M. and Pech J.C Chromoplast differentiation: current status and future prospect. Plant cell Physiology (2010 – in press

Barsan C, Paloma Sanchez-Bel P., Rombaldi C., Rossignol M., Kuntz M., Zouine M., Latché A., Bouzayen M. and Pech J.C. Metabolic and regulatory features of the tomato chromoplast revealed by proteomic analysis **,** J.Ex.Bot 61(9):2413-2431. (2010)

Barsan C., Egea I., Bian W., Purgatto E., Latché A, Chervin C., Bouzayen M. and Pech J.C Metabolic and molecular events occurring during the biogenesis of chromoplasts- Submitted to Journal of Botany, September 2010

ABSTRACT

Fruit ripening is a complex process, mainly regulated by the fruit hormone ethylene, resulting in significant metabolic and physiological changes, having as outcome seed dispersal. The most flagrant change taking place during ripening is the change in color. The organelle responsible for this is the chromoplast, the place of carotenoids accumulation. However this is not its unique role. It was found to be involved in lipid, starch, vitamins and aroma biosynthesis. Due to the fact that most proteins (95%) composing the chromoplast are codified by the nucleus knowledge on gene expression and genome sequences is not useful in the investigation of the functions of chromoplast in the synthesis of the metabolites of interest. High-throughput proteomics associated with bio-informatics was used to characterize the tomato chromoplast and to reveal its intimate structure. Analysis of the proteome of red fruit chromoplasts revealed the presence of 988 proteins corresponding to 802 Arabidopsis unigenes, among which 209 had not been listed so far in plastidial data banks. These data revealed several features of the chromoplast. Proteins of lipid metabolism and trafficking were well represented, including all the proteins of the lipoxygenase pathway required for the synthesis of lipid-derived aroma volatiles. Proteins involved in starch synthesis co-existed with several starch-degrading proteins and starch excess proteins. Chromoplasts lacked proteins of the chlorophyll biosynthesis branch and contained proteins involved in chlorophyll degradation. None of the proteins involved in the thylakoid transport machinery were discovered. Surprisingly, chromoplasts contain the entire set of Calvin cycle proteins including Rubisco, as well as the oxidative pentose phosphate pathway (OxPPP).

The analysis of the evolution of the transcriptome of chromoplastic protein-encoding genes was performed. This data confirmed the reduction of the photosynthesis and the maintenance of the Calvin cycle, and of the lipid and starch biosynthesis. Further analysis is performed showing the activity of two important actors in the aroma biosynthesis (lipoxygenase and alcohol dehydrogenase). Several proteins with possible chromoplastic location were coupled with the GFP and expressed in the single cell system. A protocol for isolating tomato fruit chloroplasts and immature chromoplasts was described along with the characterization of the plastidial fractions by confocal microscopy. The transition of the chloroplast to chromoplast is a process that was never described by means of proteomics. This work answers some questions regarding the changes that take place in the organelle, and brings novel information for the understanding of fruit ripening process.

ABSTRACT

Maturarea fructelor e un process complex, regulat in principal de hormonal vegetal etena, ce are ca efecte schimbari metabolice si fiziologice ce duc in final la dispersarea semintelor. Cea mai evidenta schimbare ce are loc in cursul coacerii este schimbarea culorii. Organelul responsabil pentru aceasta este cromoplastul, locul de stocare al carotenilor. Rolul de stocare nu este unicul rol al cromoplastelor, ele fiind implicate de asemenea in biosinteza lipidelor, amidonului si vitaminelor. Datorita faptului ca majorotatea proteinelor (95%) din componenta cromoplastului sunt codificate de nucleu, abordarea genetic nu este utila in investigarea functiilor cromoplastului in sinteza metabolitilor de interes. Strategiile de proteomica asociate cu bioinformatica au fost utilizate in caracterizarea cromoplastului de tomata si au permis descrierea compozitiei in proteine a acestuia. Analiza proteomica a cromoplastului a dus la identificarea a 987 proteine plastidiale dintre care 210 nelistate pana acum in bazele de date specifice plastelor. Plecand de la aceste date am identificate cateva trasaturi metabolice si regulatorii ale cromoplastului. Proteine implicate in metabolismul lipidelor si in transportul acestora au fost bine reprezentate, inclusiv cele implicate in calea de biosinteza a lipoxigenazei ce are ca produs final arome volatile derivate din lipide. Capaciatea de sinteza a amidonului este prezenta dar se observa prezenta enzimelor implicate in degradarea amidonului si a enzimelor de tip starch excess ceea ce poate explica absenta amidonului in fructele mature. Chromoplastul a pierdut toate proteinele implicate in biosinteza chorofilei dar similar cu cloroplastul senescent adaposteste proteine implicate in degradarea clorofilei. In mod surprinzator am regasit majoritatea proteinelor implicate in Ciclul Calvin, inclusiv Rubisco precum si calea oxidativa a pentozelor fosfatice (OxPPP), sugerand o posibila cuplare a ciclului Calvin si a OxPPP pentru re-asimilarea CO_2 si producerea de energie si putere reducatoare. Absenta aparatului de transport tilacoidal este o consecinta a dezintegrarii tilacoidelor. In concluzie, principala trasatura a metabolismului cromoplastului, pe langa inabilitatea de a sintetiza clorofila pare a fi utilizarea masinariei cloroplastice pre-existente pentru sustinerea activitatii plastidilate de baza si reorientarea metabolismului spre acumularea de carotenoizi si lipide.

A fost efectuata si o analiza a evolutiei transcriptomului asociat catorva gene ce codifica proteine plastidiale. Rezultatele au confirmat reducerea fotosintezei si mentinerea Ciclului Calvin precum si a biosintezei de lipide ai amidon. S-a analizat de asemenea si activitatea enzimatica a doi actori esentiali in biosinteza aromelor: lipoxigenaza si alcool dehidrogenaza. Cateva proteine cu locatie plastidiala posibila, dar incerta, au fost cuplate cu GFP si exprimate in sistemul single-cell. Un protocol pentru izolarea cloroplastelor si cromoplastelor immature din fructele de tomata a fost descries, impreuna cu caracterizarea plastidelor isolate prin microscopie confocala. Tranzitia cloroplastelor la cromoplaste este un proces care nu a mai fost descris pana acum din punct de vedere proteomic. Lucrarea de fata raspunde la cateva intrebari privitoare la rolul cromoplastului matur si deschide calea unui studiu mai aprofundat al evolutiei organelului, sip pe termen lung, a intelegerii mecanismelor din spatele coacerii fructelor.

RESUME

La maturation des fruits est un processus complexe, principalement régulé par l'hormone végétal éthylène, qui entraîne d'importants changements métaboliques et physiologiques, ayant pour résultat la dispersion des graines. Le changement le plus visible qui se produit pendant la maturation des fruits est le changement de couleur. L'organite responsable de ce phénomène est le chromoplaste, lieu d'accumulation des caroténoïdes. Toutefois, ce n'est pas son unique rôle. Il a été montré qu'il est aussi impliqué dans la biosynthèse des lipides, de l'amidon, des vitamines et des arômes. Parce que la plupart des protéines (95%) qui composent le protéome du chromoplaste sont codées par le noyau, l'approche génomique n'est pas suffisante pour connaître les fonctions de chromoplaste dans la synthèse des métabolites d'intérêt. La protéomique de haut débit associée à la bio-informatique a été utilisée pour caractériser le chromoplaste de tomate. L'analyse du protéome de chromoplastes de fruits de tomate rouges a révélé la présence de 988 protéines correspondantes à 802 unigènes d'Arabidopsis, dont 209 n'ont pas été répertoriés jusqu'à présent dans des banques de données plastidiales. Ces données ont révélé plusieurs caractéristiques du chromoplaste. Les protéines du métabolisme des lipides et de trafic sont bien représentées, y compris toutes les protéines de la voie de la lipoxygénase nécessaire à la synthèse des arômes volatiles dérivés de lipides. Les protéines impliquées dans la synthèse de l'amidon coexistent avec plusieurs protéines qui dégradent l'amidon. Les chromoplastes ne contiennent plus les protéines de biosynthèse de la chlorophylle mais contiennent des protéines impliquées dans la dégradation de la chlorophylle. Aucun des protéines impliquées dans le mécanisme de transport thylacoïdal n'ont été trouvées. Étonnamment, les chromoplastes contiennent l'ensemble des protéines du cycle de Calvin, y compris la Rubisco, ainsi que la voie des pentoses phosphates (OxPPP). L'analyse de l'évolution du transcriptome des gènes codant pour des protéines chromoplastiques a été réalisée. Ces données ont confirmé la réduction de la photosynthèse et le maintien du cycle de Calvin, ainsi que la biosynthèse de l'amidon et des lipides. Des analyses biochimiques complémentaires ont montré dans des chromoplastes isolés la présence d'une activité de deux enzymes importantes dans la biosynthèse des arômes (lipoxygénase et l'alcool déshydrogénase). Par ailleurs, à l'aide du couplage de protéines à la GFP et à leur expression dans des protoplastes, nous avons montré que des protéines ne présentant pas de peptide signal peuvent être localisées dans le chromoplaste. Enfin, un protocole d'isolement des plastes de fruits de tomate à différents stades de maturation a été mis au point et les fractions plastidiales ainsi obtenues ont été caractérisées par la microscopie confocale à balayage laser. La transition du chloroplaste à chromoplaste est un processus qui n'a jamais été décrit par la protéomique. Ce travail est en cours et devrait répondre à certaines questions concernant les changements qui ont lieu dans l'organite, et apporter des informations nouvelles pour la compréhension de la maturation des fruits.

GENERAL INTRODUCTION

Fruits and vegetables play a significant role in human nutrition, especially as sources of vitamins [C (ascorbic acid), A, thiamine (B_1), niacin (B_3), pyridoxine (B_6), folacin (B_9), E] and minerals, key elements for a healthy life. Due to their importance, a large number of studies were dedicated to the understanding of the ripening process and to the improvement of their organoleptic qualities in the search for fruits rich in aroma and beneficial nutrients with long shelf live.

Fruit ripening is a sophisticatedly orchestrated developmental process, unique to plants, that results in major physiological and metabolic changes, ultimately leading to fruit decay and seed dispersal (Pirrelo et al., 2010). Some of the key components participating in the control of tomato fruit ripening have been uncovered like the plant hormone ethylene that parted fruits into climacteric (ethylene responsive) and non-climacteric. Since the early 1980s, tomato has been recognized as a model system for studying the molecular basis of fleshy fruit development and unraveling the role of ethylene in controlling the ripening of climacteric fruit (Pirrelo et al., 2010).

In many fruit one of the most important and more visible changes during ripening corresponds to the loss of chlorophyll and the synthesis of coloured compounds such as carotenoids. Carotenoids accumulate in chromoplasts that are non-photosynthetic plastids often present in flowers and fruit and also occasionally found in roots and leaves. In tomato chromoplasts differentiate from chloroplasts during fruit ripening and participate in the generation of major metabolites that are essential for the sensory and nutritional quality of fruit (e.g. carotenoids, vitamins and aromas). They are also suspected to play a major role in the biogenesis of aroma volatiles. A large majority of the proteins (95%) present in the chromoplast are encoded by the nucleus and therefore imported into the organelle. The chromoplast genome encodes for only 84 proteins participating in the build-up of the chromoplast structure and in house-keeping activities. Important programmes devoted to the generation of ESTs and to the sequencing of the genome have been initiated taking tomato as a model fruit. However, for the reasons mentioned above, knowledge on gene expression and on genome sequences are of limited value for understanding the function of chromoplast in the synthesis of the metabolites of interest. Rather, high-throughput proteomics associated with bio-informatics represents the most attractive and most suitable methodology for understanding the involvement of plastid-localized proteins in such processes. Comprehensive proteome information is expected to bring new insights into processes such as intracellular

protein sorting as well as biochemical and signalling pathways. To date, the most important progress in relation to the plastid proteome has been made for chloroplasts (Kleffmann et al., 2004; Zybailov et al., 2009) and this analysis includes sub-organelle protein localization for the thylakoid and lumen, (Peltier *et al.,* 2002; Schubert *et al.,* 2002), the stroma (Peltier *et al.,* 2006), the envelope (Ferro *et al.,* 2003) and plastoglobules (Ytterberg *et al.,* 2006). Advances have also been made in protein targeting mechanisms (Zybailov *et al.,* 2008; Jarvis, 2008). The proteomes of heterotrophic plastid types have been studied less extensively and are restricted to rice etioplasts (von Zychlinski *et al.,* 2005), wheat amylopasts (Andon *et al.,* 2002; Balmer *et al.,* 2006) and tobacco proplastids (Baginsky *et al.,* 2004). An analysis of the bell pepper chromoplast identified 151 proteins using MS/MS tandem mass spectrometry (Siddique *et al.,* 2006). Protein profiling of plastoglobules from pepper fruit chromoplasts and the Arabidopsis leaf chloroplast has also been performed, yielding around 20 proteins (Ytterberg *et al.,* 2006). In the present work, we have isolated chromoplasts from ripe tomato fruit and sequenced the soluble and insoluble protein fractions using LC-MS/MS LTQ-Orbitrap technology. This proteomic study substantially enlarges the number of chromoplastic proteins identified so far and provides new information on metabolic and regulatory networks in heterotrophic chromoplasts.

The next step forward is the understanding of the chloroplast to chromoplast transition, poorly documented at the present. One third of identified proteins did not have a signal peptide predicted by TargetP. This suggests that our estimates of plastid-targeted proteins may under-represent their true number and that novel pathways and functions may still emerge. For these reasons, a full description of proteomes of chloroplast, chromoplast, even of transition organelle (immature chromoplast, is perhaps the only reliable way to provide information about quantitative and qualitative changes in proteins and pathways during chromoplast differentiation. The main challenge was the isolation of the immature chromoplasts as the ripening process is not uniform within the fruit. No information is available today about the proteome of the tomato fruit chloroplast. We report a valid isolation method for tomato fruit chloroplasts and immature chromoplasts along with proofs brought by confocal microscopy concerning the homogeneity and intactness of the samples.

BIBLIOGRAPHIC INTRODUCTION

1. Fruit types

Fruit development and ripening are unique to plants and represent an important component of human and animal diets. By anatomical definition, the fruit is a mature ovary (Giovannoni, 2004).The fruit develops mainly from the gynoecium but other organs may also participate: tepals (*Morus*), the receptacle (*Fragaria*), bracts (*Ananas*), the floral tube (*Pyrus malus*), or the enlarged axis of the inflorescence (*Ficus*). If other organs than the gynoecium participate in the formation of the fruit, it is termed a false fruit or *pseudocarp*. Fruits develop in general after fertilization but there are exceptions like certain varieties of *Musa, Citrus* and *Vitis* where the fruits have no seeds, phenomenon known as parthenocarpy (Fahn, 1967).

Evolutionary pressures have resulted in a variety of developmental manifestations of fruit tissues, resulting in structures that range in design and function from hardened fruit capsules or pods that forcefully expel seeds at maturation, to forms optimized for seed movement by wind, water, animal fur, or gravity (Giovanonni, 2004). All these types of fruits have been classifies by different methods along the years. However the simplest classification uses two criteria to part the fruits: the degree of hardness of the pericarp (fruit wall) and the ability of the fruit to dehisce or not when ripe.

1.1.Dry fruits

1.1.1. Dehiscent fruits

1.1.1.1 Fruits that develop from a single carpel

1.1.1.1.a. *Follicle*: a pod-like fruit which generally splits down the ventral side(*Delphinium, Brachychiton*).

1.1.1.1.b. *Legume*: a fruit that splits into two valves along a suture which surrounds the fruit (Leguminosae)

1.1.1.2 Syncarpous fruits-those developing from an ovary with two or more carpels

1.1.1.2.a. *Silliqua*: a pod-like fruit consisting of two carpels. The suture between the carpel's margins forms a thick rib termes *replum* around the fruit (Cruciferae)

1.1.1.2.b. *Capsule*: a fruit developing from two or more carpels and dehiscing in different ways. The portions into which fruits split are termed *valves* (*Epilobium, Hypericum, Campanula*)

1.1.2. Indehiscent dry fruits

1.1.2.1 *Achenium, achene* or *akene*: a single-seeded fruit formed by one carpel (*Ranunculus*)

1.1.2.2.*Cypsela*: a single-seeded fruit developing from an inferior ovary that originally consists of a few carpels of which all but one, in which a single ovule develops, degenerate (*Valerianella* and *Tilia*)

1.1.2.3 *Cayopsis*: a one-seeded fruit in which the seed wall is adnated to the pericarp (Gramineae)

1.1.2.4. Samara: a winged one-seed fruit (*Ulmus* and *Fraxinus*)

Several other types of dry fruit exist such as *carcerulus* - consists of several carpels and contains one or more seeds, schizocarpic fruits-they develop from multiloculate ovaries that separate when ripe into akenes, the number of which is equal to the number of carpels (Fahn, 1967).

1.2. Fleshy fruits

Several types of fleshy fruits have been described:
2.1 *Berry* or *bacca*: a fruit with a thick juicy pericarp with three distinguishable strata: the outer stratum (exocarp), the middle stratum (mezocarp), and the membranous inner stratum (endocarp). They may enclose one or many seeds (grape, tomato).

2.1.a. *Hesperidium* the fruit of *Citrus*

2.1.2. False fruits: they develop from inferior ovaries and differ from typical false fruits in that the extracarpellary parts contribute only a small part in the construction of their pericarp (*Coffea, Sambucus, hedera, Cucumis* and *Musa*)

2.1.2.c. *Drupe*: has a thick and hard endocarp (*Prunus, Mangifera, Pistacia*)

2.1.2.d. *Aggregate fruits*: the carpels and apocarpous gynoecium ripen individually but in the course of ripening the individual fruits of a flower aggregate to form single units (*Rubus*) (Fahn, 1967)

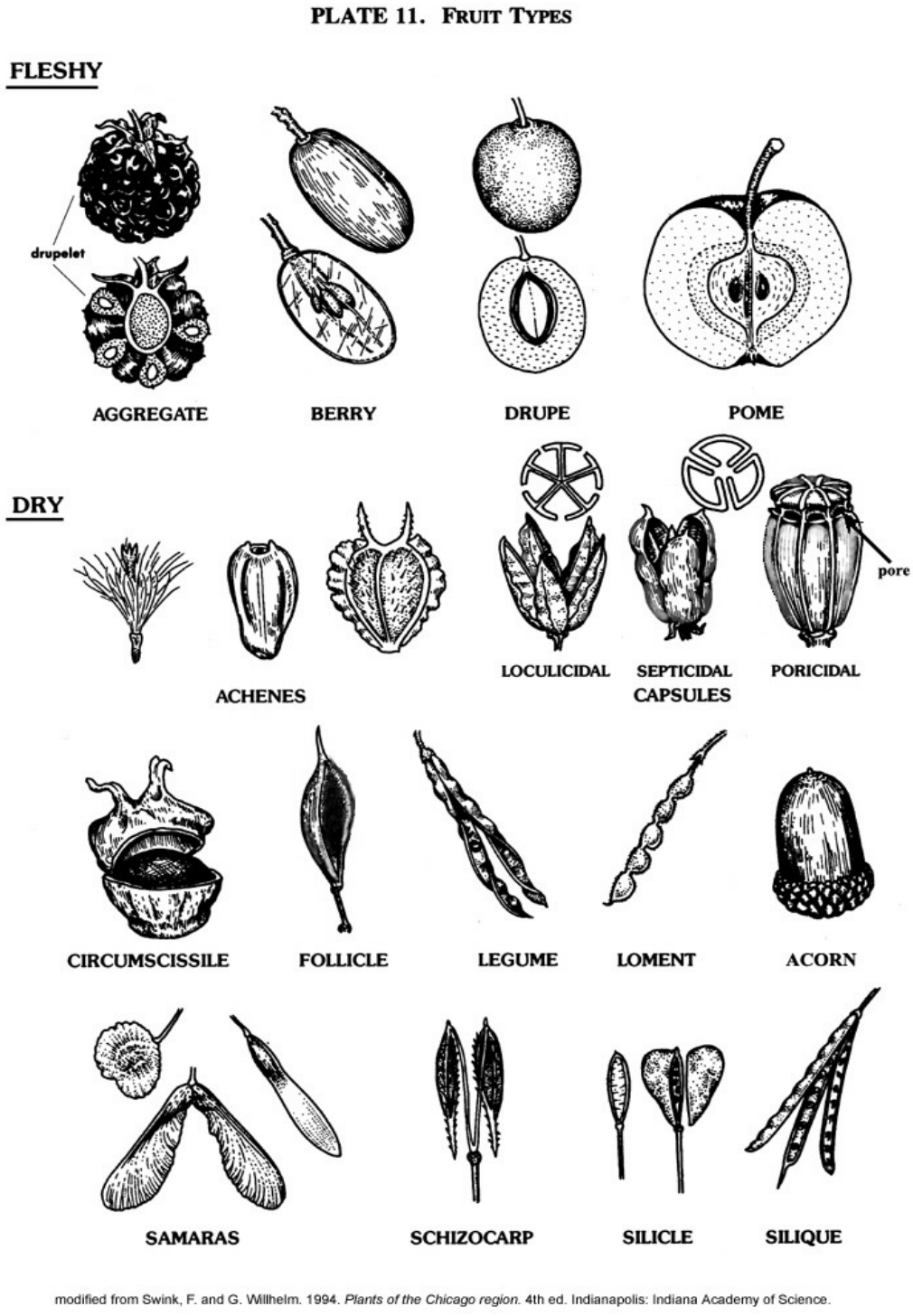


Figure 1: Fruit types (www.vplants.org/plants/glossary/plate_all.html.)

2. *Fruit ripening*

Ripening can be defined as a series of coordinated metabolically events that start at a specific developmental stage. Fruit development and ripening are specific to plants and have a major importance in human and animal diet. Although dehiscent and dry fruit types (e.g., cereals) represent the majority of plant species, fruit developmental studies to date have focused primarily on fleshy species because of their importance in the human diet (Giovannoni, 2004). The ripening of dry fruits is a complex process defined by changes in flavor, firmness, aroma, color and associated with fruit abscission; a process depended by seed development (Giovannoni, 2001). In spite of its complexity the ripening process was studied in the early 1920's when a burst in selling, stocking and fruit transport occurred. Fruit breathing was one of the first studied processes and lead to the early definition of climacteric fruits. Climacteric fruits such as apple, banana, tomato and others have a respiration peak during ripening while fruits that do not display this peak such as strawberry, grapes, oranges are called non-climacteric.

2.1. Ripening of climacteric fruits

The majority of fruit quality attributes are elaborated during the ripening process. These traits correspond to visual, chemical and structural modifications that ultimately make fruit edible and attractive for consumption. Because these changes are crucial for the final sensory and nutritional qualities of the fruit, they have received great attention from scientists and breeders and studies have been directed toward a better understanding of their physiological, molecular and genetic basis. Among all the aspects contributing to fruit quality, changes in texture, aroma, volatile production and pigment accumulation have been most extensively studied in the tomato (fig.1). Efforts in this area have first concentrated on the isolation and characterization of genes and enzymes that participate directly in the above mentioned biochemical and physiological changes. Thereafter, attempts were made to unravel the regulatory mechanisms controlling these complex processes. Studies of secondary metabolites accumulating during tomato fruit ripening were further prompted by health claims concerning these compounds, even though direct and clear evidence of their positive impact on human health is still lacking.

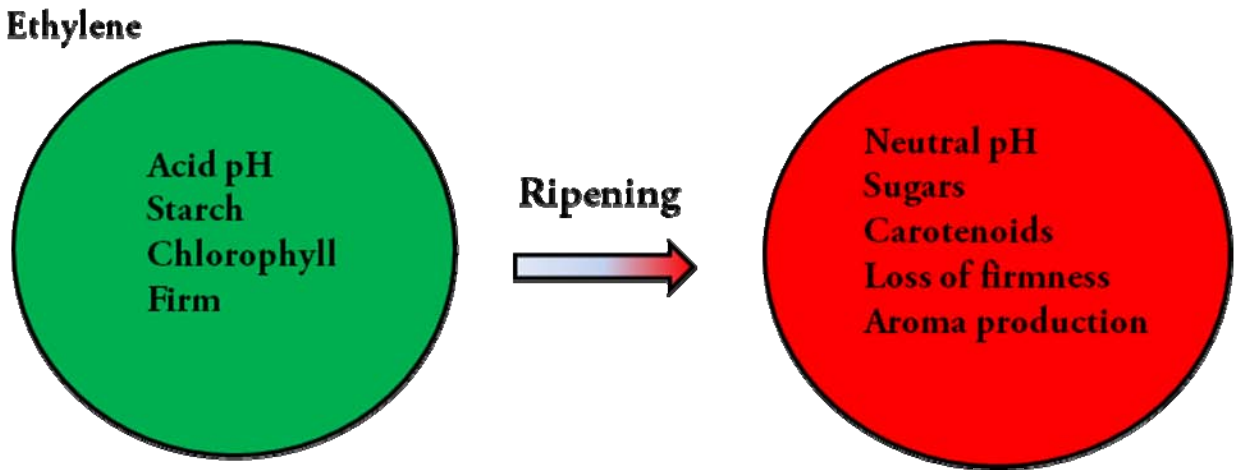


Figure 1 Main events taking place in climacteric fruits during ripening

2.1.1. Cell wall softening

An important process that is taking place during fruit ripening is the *cell wall softening*. The cell wall (figure 2) acts as a cellular exoskeleton that encases plant cells, giving them shape and mechanical stability, gluing them together, restraining their growth, and protecting them from assaults by pathogens and the environment. The plant cell wall is a complex and heterogeneous layer, typically between 0.1and 1µm thick, consisting of cellulose microfibrils embedded in a highly hydrated matrix of hemicelluloses and pectins, with smaller quantities of structural protein intercalated in the matrix. Cellulose is made up of 1→4 linked β-D-glucans tightly packed into long, crystalline microfibrils that wind around the cell. Hemicelluloses anchor the microfibril in the matrix by bonding noncovalently to the surface of the microfibril and perhaps by becoming physically entrapped in the microfibril as it is formed by synthase complexes in the plasma membrane. Pectins make up a coextensive hydrophilic phase with gel-like properties; situated in the space between microfibrils, pectins prevent aggregation and collapse of the cellulose network. For years it was hypothesized that enlargement of the growing wall—like the softening of fruit—was primarily based on the activity of wall hydrolases or transglycosylases (Showalter, 1993).

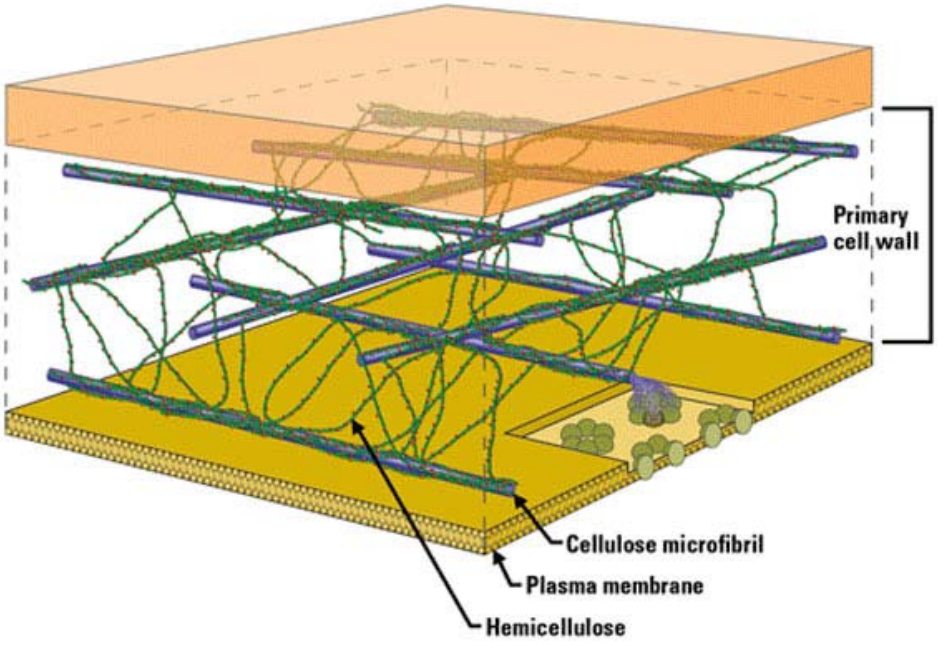


Figure 2: Structure of the primary plant cell wall, showing major structural polymers and their likely arrangement in the wall wall (**Source:** © United States Department of Energy Genome Programs/ genomics.energy.gov.)

Fleshy fruits become softer in the later stages of ripening. This aspect of fruit ripening was long considered to be mediated by pectinases and other wall hydrolases that degrade the major structural polymers of the wall. This idea, however, lost much of its lustre in the last decade when experiments with transgenic tomatoes showed that expression of these hydrolytic enzymes could be genetically altered without major effects on fruit softening. Such experiments have, one by one, downgraded the major candidates from the list of suspected fruit-softening enzymes and clouded the view that fruit softening is the primarily the result of wall hydrolysis (Cosgrove, 1997). Polygalacturonase (PG) has been the most widely studied cell wall hydrolase. It catalyses the hydrolytic cleavage of a-(1-4)-galacturonan linkages and is responsible for the change in pectin structure associated with the ripening of many fruits (Pirello et al., 2009). The high-level extractable endo-PG activity increased in parallel with the ripening process. These observations led to the pursuit of the tomato endo-PG gene and the hypothesis regarding the role of PG in ripening-related textural modifications. Gene isolation, and the subsequent functional characterization of tomato fruit PG in transgenic plants, indicated that PG activity alone is not sufficient to significantly impact texture; thus it is likely to function in concert with additional

factors. Enzymes in addition to PG that are involved in cell wall metabolism have been identified in ripening fruit. Pectin-methyl esterase (PME) showed activity through fruit development and its possible function may be to increase accessibility of PG to its pectin substrate (Givannoni, 2001). β-galactosidase and α-galactosidase activities were the most readily detectable and were found to increase markedly during berry softening. β -galactosidase may be the enzyme responsible for the loss of galactan from cell walls as berries soften. Pectin methylesterase activity remained relatively low during berry development, consistent with the observation that the degree of esterification of pectins remained roughly the same during berry softening. Little or no activity was detected for polygalacturonase, galactanase, cellulase or xyloglucanase in ripening berries (Nunan, 1999). An expansin mRNA is specifically and abundantly expressed in ripening fruit and it was suggested that expansin proteins might contribute to cell wall disassembly during fruit ripening (Rose et al., 1997).

The involvement of expansins in fruit ripening was surprising because these proteins were not known to possess cell wall hydrolytic activity and because they were mainly known as catalysts of plant cell enlargement. Growing plant tissues characteristically possess a property known as "acid growth":plant cells to extend rapidly when incubated in acidic buffers (pH <5.5) the extension, stopped at neutral pH but when switched to a pH 4.5 buffer, a rapid and irreversibly by extension by a process of polymer creep was observed (Cosgrove; 1989).

Cloning and sequence analysis showed expansins to be a novel gene family common to the two major branches of angiosperms (monocotyledons and dicotyledons) (Cosgrove, 1997).

2.1.2. Color change

Biosynthesis of a large variety of secondary metabolites is one of the most remarkable features of ripe fruit, and in the case of tomato, red pigment accumulation is the most obvious change during the ripening process. The characteristic color of ripe tomato fruit is caused by accumulation of lycopene and β-carotene, concomitantly with the decrease in chlorophyll content during the transition from chloroplast to chromoplast. As photosynthetic membranes are degraded, chlorophyll is metabolized and carotenoids, including β-carotene and lycopene accumulate. At the breaker stage of ripening, the red color of lycopene begins to appear, the chlorophyll content decreases and the organoleptic properties of the fruit change. (Bramley, 2002)

The regulation of carotenoid biosynthesis during ripening is due, at least in part, to ripening - related and ethylene-inducible gene expression in both tomato and melon (Giovannoni, 2001). Carotenoids are isoprenoid molecules common to all photosynthetic tissues. They are divided into the hydrocarbon carotenes such as lycopene and β-carotene or xantophylls, typified by lutein (Bramley, 2002) (fig 3.).

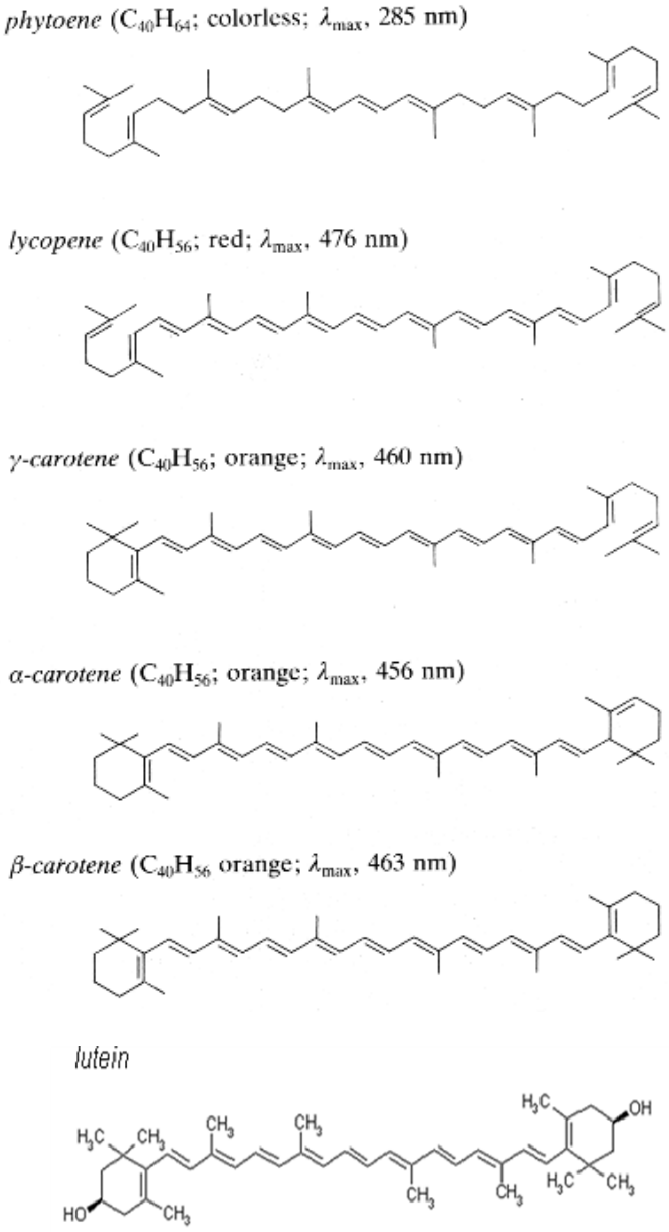


Figure 3 chemical formulas of the main carotenoids: phytoene, lycopene, γ-carotene,α-carotene, β-carotene and lutein

Carotenoid biosynthesis (fig.4) is a complex pathway distributed in two main steps and involving a large number of enzymes.

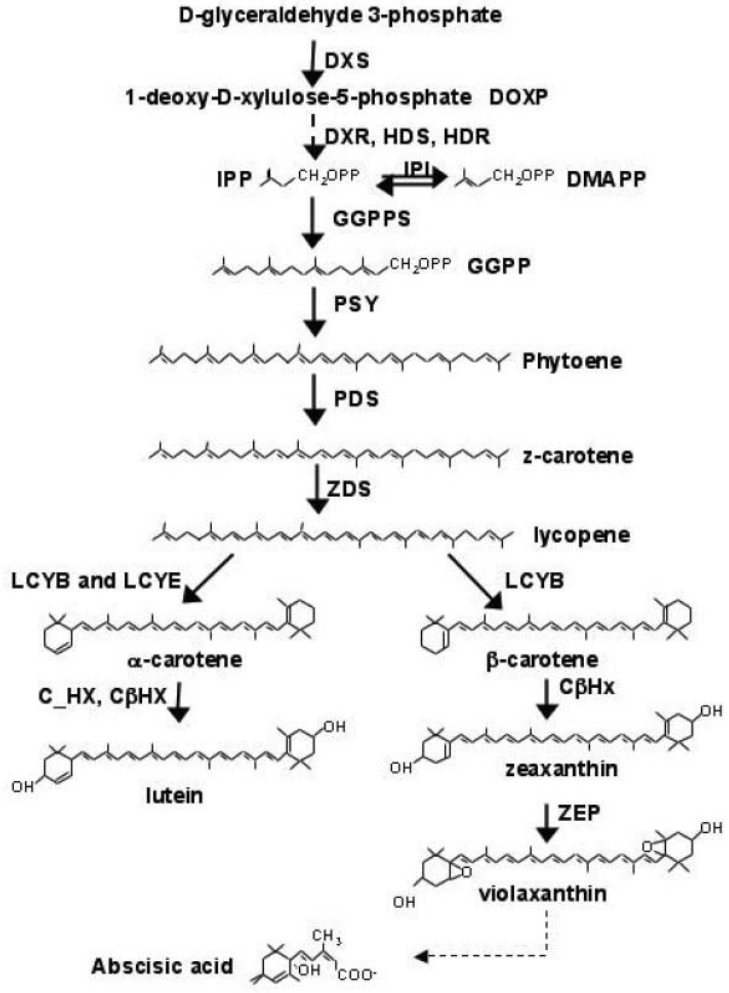


Figure 4. Schematic representation of carotenoid synthesis in plants. The isopentenyl pyrophosphate (IPP) is synthesized in plastids through the non-mevalonate route, and begins with the synthesis of DOXP catalyzed by DOXP synthase (DXS). The other enzymes that participate in the biosynthesis of carotenoids and abscisic acid are: isopentenyl pyrophosphate synthase (IPI), geranylgeranyl pyrophosphate synthase (GGPPS), phytoene synthase (PSY), phytoene desaturase (PDS), z-carotene desaturase (ZDS), carotene isomerase (CRTISO), lycopene ε cyclase (LCYE), lycopene β cyclase (LCYB), β-carotene hydroxylase (CβHx), ε-carotene hydroxylase (CεHx) and zeaxanthin epoxidase (ZEP). The name and structure of the synthesized carotenes and xanthophylls are included (Stange et al., 2008)

In the early step, also known as the non-mevalonate pathway, the hydroxyethyl thiamine is condensate of into 1-deoxy-D-xylulose 5-phosphate by the DOXP synthase (1-deoxy- D-xylulose-5-phosphate) (Lange et al., 1998, Rohmer et al., 1999). In the next step, named the

isoprenoid pathway, the enzyme phytoene synthase (PSY) catalyses the condensation of two molecules of geranylgeranyl pyrophosphate (GGPP) into phytoene (Cunningham et al., 1998), the immediate precursor of lycopene, whose accumulation is correlated with the up-regulation of isoprenoid genes such as DOXP synthase, suggesting a crucial role for the nonmevalonate pathway in lycopene biosynthesis during fruit ripening (Lois et al., 2000). During ripening genes encoding for enzyme involved in phytoene formation and desaturation (phytoene synthase (PSY1) and phytoene desaturase (PDS)), are also up-regulated (Fraser et al., 1994, , Pecker et al., 1992, Giuliano et al., 1993, Corona et al., 1996), leading to lycopene formation. Concomitantly lycopene cyclization is impared leading to its accumulation. This was reflected in a strong down-regulation of lycopene cyclase genes (LCY-b and LCY-e) during ripening (Pecker et al., 1996, Ronen et al., 1999). The inhibition of lycopene cyclization induced an increase in PDS and PSY-1 expression, suggesting the existence of an autocatalytic synthesis of lycopene (Giuliano et al., 1993, Corona et al., 1996). Red light treatment stimulated lycopene accumulation that was found to be under the dependence of fruit localized phytochrome (Alba et al., 2000).

2.1.3. Ethylene, key hormone for climacteric fruit ripening

Based on their type of ripening mechanisms fruits can be divided into climacteric and non-climacteric, (Biale et al., 1964). Climacteric fruits present a peak in respiration and a concomitant burst of ethylene during maturation, process that does not take place in the nonclimacteric fruit type. This category of fruit includes tomato, banana, pears and apple; all of them need an ethylene burst for normal ripening. Corroboratively delayed or suppressed ripening is observed in ethylene-suppressed transgenic plants (Hamilton et al., 1990, Oeller et al., 1991, Ayub et al., 1995). Ethylene is a simple gaseous molecule that plays a key role in many processes, including seed germination, leaf senescence, abscission, responses to stresses and fruit ripening (Pirrelo et al., 2009).The critical role of ethylene in coordinating climacteric ripening at the molecular level was first observed via analysis of ethylene-inducible ripening-related-gene expression in tomato (Givanonni, 2001). While fruit development from fruit set through ripening involves a number of plant hormones, the phytohormone ethylene was first identified as the key regulator of tomato fruit ripening. Inhibition of ethylene with inhibitors, transgenic approaches or in mutants blocks ripening. The tomato *never-ripe* mutation blocks fruit ripening and is

insensitive to ethylene. The mutated gene is similar to the ethylene receptor isolated from Arabidopsis, suggesting that *never-ripe* is an ethylene receptor mutant. *NR* mRNA is not expressed until the mature green stage, suggesting that lack of this ethylene receptor might be related to the lack of competence to respond to ethylene at earlier stages (Barry et al., 2006). Exogenous ethylene accelerates ripening. Environmental factors to which fruits are exposed during storage and postharvest ripening have also the potential to greatly influence the level of ethylene biosynthesis. For example, low temperatures are generally applied for extending the storage life of fruit but they can also accelerate ethylene synthesis and induce premature ripening in temperate fruits such as pears. Changes in gas composition in modified and controlled atmosphere storage can have dramatic effects on the biosynthesis of ethylene and its precursors (Lelièvre et al., 1997).

Ethylene biosynthesis in higher plants originates from S-adenosyl-methionine (SAM) and comprises two steps catalyzed by ACC synthase (ACS) and ACC oxidase (ACO), the latter converting 1-aminocyclopropane-1-carboxylic acid (ACC) into ethylene (Yang et al., 1984, Yoo et al., 2008) (fig. 5). Genes encoding these two enzymes undergo important regulation during the process of fruit ripening.

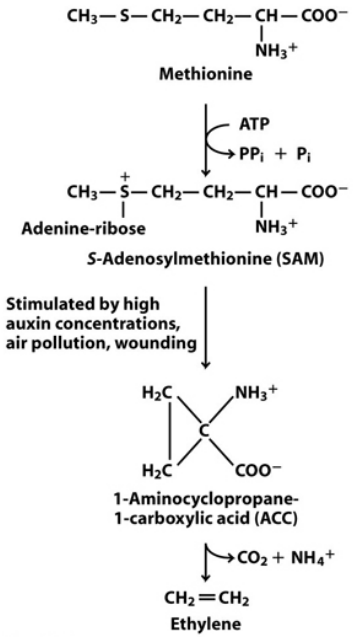


Figure 5: Ethylene biosynthesis

Two distinct systems of ethylene biosynthesis have been proposed to take place during fruit development: the auto-inhibitory and the autocatalytic ethylene production (Lelievre et al., 1997). The auto-inhibitory ethylene production is responsible for producing basal ethylene levels that are detected in all vegetative tissues and in preclimacteric stages of climacteric and non-climacteric fruit development and relies on the expression of ACS1A and ACS6 (Barry et al., 2000) During climacteric burst there is an autocatalytic production of ethylene initiated and maintained by the ethylene dependent ACS2 (Barry et al., 2000). In non-climacteric fruits (pineapple, lemon, cherry, …) ripening is generally considered as an ethylene - independent process, although some recent results suggest a role of ethylene in ripening this type of fruit (Chervin et al., 2004, Trainotti et al., 2005).

2.1.4. Regulation of volatile formation during tomato fruit ripening

Even if the overall sensory quality of fruit is greatly influenced by aroma volatiles the most prevalent compounds that are essential for typical aroma of ripe tomato fruit are still largely unknown. Only a few volatile compounds out of 400 in ripe tomato but have been considered to play a major role in tomato flavor. Tomato volatile compounds are usually grouped into five main classes (Baldwin et al., 2000, Buttery et al., 1993, Canoles et al., 2006, Oke et al., 2003, Chen et al., 2004, Speirs et al., 1998, Tieman et al., 2006, Simkin et al., 2004, Lewinsohn et al., 2001) based on their metabolic origin (Table 1)

Table 1. Metabolic origin of main volatile compounds involved in tomato fruit flavor (Pirello et al., 2009)

Pathway	Component	Enzymes
Fatty acids oxidation	cis73-hexenal	Phospholipase
	Hexanal	Lipoxygenase
	1-penten-3-one	Hydroperoxydelyase
	Trans-2-hexenalTrans-2-pentenalpentenol1-penten-3-oltrans-2-heptenal2-isobutylthiazole	Alcoholdehydrogenase
Aminoacids	2-phenylethanol	Aminoaciddecarboxylases
	3-methyl-butanol1-nitro-3-methyl-butane2+3-methyl-butanal	(AADC1A,AADC1B,AADC2)
Carotenoidrelated	6-methyl-5-hepten-2-one	Carotenoidcleavage
	Geranyl-acetonePseudoionone	dioxygenase1
	b-ionone	
Terpenepathway	Geranial Linalool	Linalool synthase
	Neral	
Shikimatepathway	Methylsalicylate	Unknown

The lipid-derived volatiles represent the bulk of aroma volatiles in tomato and are generated by the lipoxygenase (LOX) pathway, that appears to be located in the plastid since a

natural mutation in a chloroplastic w-3-fatty acid desaturase gene that resulted in a deficiency in linolenic acid caused profound changes in the volatile profile of tomato (Canoles et al., 2006). The pathway is composed by the action of phospholipase, lipoxygenase, hydroxyperoxidelyase and alcohol dehydrogenase, enzymes encoded by multigene families. The down-regulation of only one of the five LOX of tomato, LOXC, did not result a significant reduction in the level of flavor volatiles such as hexanal, hexenal and hexenol (Chen et al., 2004). Other important components of the aroma of tomato fruit are the amino acid-derived volatiles .The identification of the gene encoding the enzyme responsible for the decarboxylation of phenylalanine represents a significant step forward towards the understanding of this metabolic pathway (Tieman et al., 2005). Down-regulation of the phenylalanine gene led to reduced emissions of phenylacetaldehyde and phenylethanol in transgenic tomatoes, its overexpression in tomato leading to an increase up to 10-fold the quantities of phenylethanol, phenylacetaldehyde, phenylacetonitrile and 1-nitro-2-phenylethane. These compounds can exert a dual effect: at high concentrations the pungent aroma of phenylacetaldehyde has a nauseating and unpleasant odor while at low concentrations, phenylethanol and phenylacetaldehyde are associated with pleasant sweet flowery notes (Tadmor et al., 2002). Carotenoid-derived volatiles play an important role in tomato flavor. The biosynthetic route was discovered by Simkin et al., 2004 who demonstrated, by both heterologous expression in Escherichia coli and down-regulation in tomato plants, that the carotenoid cleavage dioxygenase 1 genes contribute to the formation of b-ionone, pseudoionone and geranylacetone. Tomato produces low amounts of terpene volatiles. Expressing the *Clarkia breweri* linalool synthase gene under a fruit-specific promoter in the tomato was reported to result in a strong stimulation of the production of linalool and of 8-hydroxy-linalool, probably as a result of the presence in the tomato of a P450 enzyme capable of hydroxylating linalool (Lewinsohn et al., 2001).

In ripe tomato many volatile compounds are present in a conjugated form, linked to glycosides to form non-volatile precursors that could be as important in quantity as the free fraction (Ortiz-Serrano et al., 2007). The proposed mechanism governing, in vivo, the release of volatiles from the bound fraction is supposed to occur by the action of endogenous b-glucosidases, preferentially upon cell disruption. An increase in the production of aroma volatiles has been observed upon tissue disruption. Glycoside derivatives are synthesized by glycosyltransferases

(GTs), enzymes encoded by a very large gene family but so far, data on which GT genes are specifically involved in the formation of conjugated volatiles are not available.

Tomato as model plant

Tomato is a well-established model organism for the study of many biological processes. It presents multiple advantages that make it the universal model for the study of climacteric fruits Tomato is an attractive model species because of the availability of a wide range of well-characterized spontaneous or induced mutants; ease of genetic transformation and manipulation and the existence of a dwarf varieties (Pirello et al., 2009). All data generated with this model is applicable for all the other members of the *Solaneacea* family (potato, eggplant) who are largely consumed by the public. Tomato offers several features that enable studies on the development and ripening of fleshy fruit and on many plant–pathogen interactions that affect economically important plants. It presents also more practical advantages: a diploid genome, with a relatively small size (n=12) compared to that of the other species of agronomical interest., a relatively short reproductive cycle (3-4 generation per year) and a large number of information at the genetic level – a genetic map having more than one million markers separated in average by less that 1cM (Tanksley, 1996). Its moderately sized genome (950 Mb with hundreds of mapped traits and molecular markers), tolerance to inbreeding, amenability to genetic transformation, and diversity of secondary metabolism make tomato an excellent platform for genetic and molecular research Compared with other commercial crop plants, a relatively large number of single-gene-determined traits have been described in tomato, with an estimate of 1200 available monogenic traits. Map-based cloning recently resulted in the first cloned quantitative trait loci in tomato. It is predicted that the tomato genome encodes 35,000 genes sequestered largely in the euchromatic regions, corresponding to less than one-quarter of the total DNA in the tomato nucleus (Mathews et al., 2003). The adaptation of a range of technological tools (e.g. microarray) and the generation of new biological resources on the tomato (e.g. EST database, TILLING resources, genetic and physical maps) have led to a step forward on the understanding of the molecular mechanisms underlying the ripening process. (Pirello et al., 2009).

3. Plastids

Plastids are a family of interrelated organelles of various forms found in plants and algae. They are involved in numerous metabolic pathways including nitrate assimilation, starch

metabolism and fatty acid biosynthesis and thus are vital to plant cell functionality (Waters et al., 2004). Plastids develop and differentiate into specialized plastid types that can be distinguished by their structure, pigment composition and functional properties. Examples of specialized plastids include chloroplasts in photosynthetically active leaf tissues, chromoplasts in fruit and petals, amyloplasts in roots and storage tissues (fig. 1).

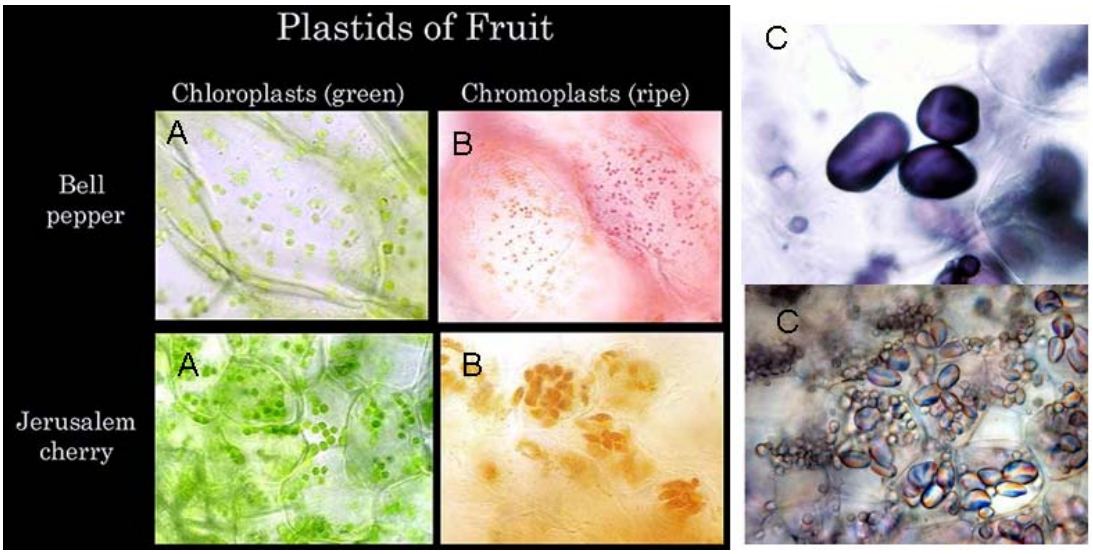


Fig. 1: Chloroplasts (A), Chromoplasts (B); from Bell pepper and Jerusalem cherry; and amyloplasts (C) from potato tissue (http://botit.botany.wisc.edu)

Based on their energy metabolism, plastids can be distinguished as photosynthetic (autotrophic) and non-photosynthetic (heterotrophic). Photosynthetic chloroplasts synthesize sugar phosphates that are metabolized by oxidative metabolism to NADPH and ATP. Non-photosynthetic plastid types import sugar phosphates and ATP from the cytosol which is necessary to sustain their anabolic metabolism (Siddique et al., 2006).

It is now clear that non-green plastids, although devoid of the photosynthetic capability, are metabolically active forms of plastids, often involved in the biosynthesis of many aromatic compounds and essential oils. This holds true for chromoplasts which are often formed from redifferentiating chloroplasts and defined as plastids lacking chlorophylls which accumulate pigments of the carotenoid class (Marano et al., 1993, Camara et al., 1995).

CHAPTER I

Chromoplast Differentiation: current status and Perspectives

Cristina BARSAN [1,2,a] , Isabel EGEA[1,2,a], , Wanping BIAN [1,2], Eduardo PURGATTO[3], Alain LATCHE[1,2], Christian CHERVIN[1,2], Mondher BOUZAYEN[1,2] and Jean-Claude PECH[1,2]

[a] Participated equally to the work - **Plant Cell Physiology, 2010, in press**

ABSTRACT

Chromoplasts are carotenoid-accumulating plastids conferring the color to many flowers and fruits as well as to some tubers and roots. Chromoplast differentiation proceeds from pre-existing plastids, most often chloroplasts. One of the most prominent changes is the re-modeling of the internal membrane system associated with the formation of carotenoid-accumulating structures. During the differentiation process the plastid genome is essentially stable and transcriptional activity is restricted. The build-up of the chromoplast for giving its specific metabolic characteristics is essentially dependent upon transcriptional activity of the nucleus. Important progress has been made in terms of mediation of the chloroplast-to-chromoplast transition with the discovery of the crucial role of the *Or* gene. In this paper, we review recent developments in the structural, biochemical and molecular aspects of chromoplast differentiation and also consider the reverse differentiation of chromoplasts into chloroplast-like structures during the re-greening process occurring in some fruit. Future perspectives towards the full understanding of chromoplast differentiation include the in depth knowledge of the changes occurring in the plastidial proteome during chromoplastogenesis, the elucidation of the role of hormones and the search for signals that govern the dialog between the nuclear and the chromoplastic genome.

During evolution higher plants have adopted strategies to attract insects and mammals so as to facilitate flower pollination and seed dispersal. One of these strategies has been the development of bright colors most often within a type of plastids named chromoplasts. Chromoplasts are responsible for yellow, orange or red colors of many flowers and fruits. They are also present in some roots, such as carrot, or tubers such as sweet potatoes. Plastids are typical organelles unique to lower and higher plants that originate from the endosymbiotic integration of a photosynthetic

prokaryote, cyanobacterium, into a eukaryotic ancestor of algae. The ancestors of plastids, chloroplasts, have diversified into a variety of other plastid types, including chromoplasts to carry-out specialized functions in non-photosynthetic organs (Pyke 2007). Among non photosynthetic plastids, chromoplasts have received most attention as they accumulate pigments that are essential for the sensory quality of horticultural products. Most of the pigments present in chromoplasts being carotenoids, the biochemistry and molecular biology of chromoplast differentiation has been largely devoted to the biochemistry and molecular biology of carotenoid formation (Camara et al., 1995, Bramley 2002). However, despite strong specialization, non-photosynthetic plastids also carry out many other functions either specific or remnant of chloroplastic functions in flowers (Tetlow et al., 2003) and fruits (Büker et al., 1998, Bouvier and Camara 2007). Biochemical and structural events during chromoplast differentiation has been reviewed, either specifically (Marano et al., 1993, Ljubesic et al., 1991) or within general papers on non-green plastids (Thomson and Whatley 1980) and on plastid differentiation (Waters and Pyke 2004; Lopez-Juez 2007). Since then, novel information on the specific metabolic capacities of chromoplasts has been generated using high-throughput transcriptomic (Kahlau and Bock 2008) and proteomic approaches (Siddique et al., 2006, Barsan et al., 2010).

Introduction

During evolution higher plants have adopted strategies to attract insects and mammals so as to facilitate flower pollination and seed dispersal. One of these strategies has been the development of bright colors most often within a type of plastids named chromoplasts. Chromoplasts are responsible for yellow, orange or red colours of many flowers and fruits. They are also present in some roots, such as carrot, or tubers such as sweet potatoes. Plastids are typical organelles unique to lower and higher plants that originate from the endosymbiotic integration of a photosynthetic prokaryote, cyanobacterium, into a eukaryotic ancestor of algae. The ancestors of plastids, chloroplasts, have diversified into a variety of other plastid types, including chromoplasts to carry-out specialized functions in non-photosynthetic organs (Pyke 2007). Among non photosynthetic plastids, chromoplasts have received most attention as they accumulate pigments that are essential for the sensory quality of horticultural products. Most of the pigments present in chromoplasts being carotenoids, the biochemistry and molecular biology of chromoplast differentiation has been largely devoted to the biochemistry and molecular biology of carotenoid formation (Camara et al. 1995, Bramley 2002). However, despite strong specialization, non-

photosynthetic plastids also carry out many other functions either specific or remnant of chloroplastic functions in flowers (Tetlow et al. 2003) and fruits (Büker et al. 1998, Bouvier and Camara 2007). Biochemical and structural events during chromoplast differentiation has been reviewed, either specifically (Marano et al. 1993, Ljubesic et al. 1991) or within general papers on non-green plastids (Thomson and Whatley 1980) and on plastid differentiation (Waters and Pyke 2004; Lopez-Juez 2007). Since then, novel information on the specific metabolic capacities of chromoplasts has been generated using high-throughput transcriptomic (Kahlau and Bock 2008) and proteomic approaches (Siddique et al. 2006, Barsan et al. 2010).

This review focuses on the recent data on the structural and molecular events occurring during the differentiation of chromoplasts to better understand how chromoplasts acquire their specific metabolic characteristics.

1. Diversity of chromoplast structures

There is a great variation in the morphology of chromoplasts, particularly in the structures that contain carotenoids. A classification into globular, membranous, tubular, reticulo-globular and crystalline has been proposed, although there is generally more than one type of pigment-containing bodies in a chromoplast (Ljubesic et al., 1991). Reticulo-tubular structures made of a reticulum of tubules are abundant in saffron, bananas and *Cucurbita maxima* but vesicles and globules also co-exist (Caiola and Canini 2004). Some chromoplasts accumulate carotenoids as large crystals inside the lumina of thylakoid-like structures such as in carrot roots and daffodil petals and plastoglubuli containing small amounts of carotenoids are also present although in small size and number (Kim et al., 2010). Mango fruit chromoplasts contain large and numerous gobules as well as a network of tubular membranes. They can be considered of both the globular and reticulo-tubular type (Vasquez-Caicedo et al., 2006). Tomato fruit accumulates carotenoids, predominantly under the form of lycopene crystalloids in membrane-shaped structures (Harris and Spurr 1969). Chromoplasts of red pepper are characterized by a large number of globules with fibrillar extensions of carotenoid (Laborde and Spurr 1973). Different types of chromoplasts may co-exist in the same organ. For instance, in *Thunbergia alata* flowers, mesophyll cells harbor chromoplasts of the tubulous type almost exclusively, while adaxial epidermial cells contain, in addition to the tubules, membrane and tubular reticulum structures (Ljubesic et al., 1996). A detailed supramolecular organization of the carotenoid-protein bodies of red pepper has

been described by Deruere et al., (1994) showing that the carotenoids, in association with tocopherols and quinones are sequestred in the central core and are surrounded by a layer of polar lipids, which in turn are surrounded by an outer layer of the plastoglobulin, fibrillin. In fact fibrillin which is highly expressed in ripening fruit allows the sequestration of lycopene under the form of crystals within membrane structures. The sequestration prevents the otherwise detrimental effects of excess of carotenoids on cellular functions. Fibril initiation occurs in the plastoglobule. In chloroplasts where the level of carotenoids is low, the lipid-protein ratio is sufficient for the sequestration of carotenoids. The type of carotenoid-containing bodies therefore depends upon the lipid to protein ratio and the presence of proteins facilitating the assembly of carotenoids, such as fibrillin.

Chromoplasts that accumulate pigments during fruit ripening and flower development are functionally different from senescence-derived plastids. The yellow color of senescent plastids is due to the disappearance of chlorophyll and retention of carotenoids in the absence of *de novo* carotenoid biosynthesis. In addition, contrary to chromoplasts, they undergo an extensive loss of plastidial DNA and are designed as gerontoplasts (Matile 2000).

2. Changes in structure, morphology and composition of the plastid during chromoplast formation

i) Changes in morphology and chlorophyll-carotenoids balance during chromoplast differentiation

Insights into the morphology of plastid differentiation, chlorophyll breakdown and carotenoid accumulation has been provided by confocal microscopy coupled with the plastid-located green fluorescent protein (GFP; Köhler and Hanson 2000, Waters et al., 2004, Forth and Pyke 2006). Pericarp cells in young green tomato fruit have a large number of regular-sized plastids containing both chlorophyll and GFP, visualized by red auto-fluorescence and green fluorescence, respectively. As fruits ripen, the red fluorescence of plastids decreases in relation with chlorophyll degradation. Fully ripe pericarp cells possess a large population of chromoplasts, appearing in green due to the exclusive fluorescence of GFP in the absence of chlorophyll (Forth and Pyke 2006). The plastid size varies from the mature-green to the fully ripe stages, chromoplast being smaller than chloroplasts. At the breaker stage, plastids show

considerable intracellular variability in size and differentiation status. The chloroplast-chromoplast transition events are presumably not simultaneous throughout the fruit or even within a cell, leading to a heterogeneous population of plastids. In addition, there are consistent differences in plastid size and appearance between inner mesocarp and outer mesocarp cells of tomato fruit. Chromoplasts of the outer mesocarp have an oblong, needle-like appearance, whereas chromoplasts in the inner mesocarp are much larger and have an ovoid shape (Waters et al., 2004).

The transition chloroplast-to-chromoplast can be visualised during the ripening process by exploiting the auto-fluorescence of chlorophyll and carotenoids of purified plastid fractions (Fig. 1). At the mature green stage all plastids are chloroplast and the emitted fluorescence gives a red color due to the dominance of chlorophyll (Fig. 1A). At the breaker stage the population of plastids is highly heterogeneous, but by using adapted isolation procedures, intermediate chloro-chromoplasts can be obtained containing both chlorophyll and carotenoids so that the emitted fluorescence gives a yellowish color due to the merging of red (chlorophyll) and green (carotenoid) fluorescence (Fig. 1B). At the fully ripe stage only fully developed chromoplasts are present and appear in green corresponding to the auto-fluorescence of carotenoids (Fig. 1C).

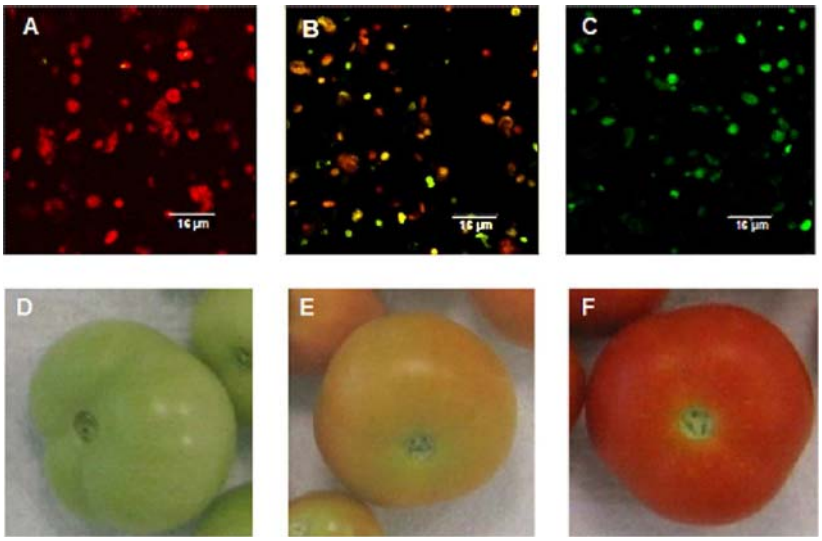


Figure 1. Confocal images of chloroplast (A), chloroplast initiating transition (B) and mature chromoplast (C) suspensions isolated from mature green (D), breaker (E) and fully ripe (F) tomatoes. Images are overlays of chlorophyll autofluorescence and carotenoid autofluorescence emitted at wavelengths between 740 and 750 nm (red) and between 500 and 510 nm (green), respectively, when they are excited using the 488 nm line from the argon laser. Structures containing mainly chlorophyll appear red, those containing only carotenoid appear green and those containing both chlorophyll and carotenoid appear orangey red/yellow. Scale bars=16 µm.

ii) Pre-existing plastids from which chromoplasts originate

Different forms of plastids can be generated by inter-conversions of pre-existing plastid types. A cycle of plastid development interrelationships has been suggested (Whatley 1978). Chloroplast differentiation from proplastids, under the control of light is one of the best-known inter-conversion (Lopez-Juez and Pyke 2005). Chromoplasts may arise directly from proplastids e.g. in carrot roots (Ben-Shaul and Klein 1965) or indirectly from chloroplasts e.g. in ripening fruit (Bathgate et al., 1985) or from amyloplasts e.g. in saffron flowers (Caiola and Canini 2004) or tobacco floral nectaries (Horner et al., 2007). An interesting example of plasticity exists in *Arum italicum* berry fruit where the various steps of maturation and ripening are associated with a sequence of transitions involving amyloplast, chloroplast and chromoplast (Bonora et al., 2000).

Analysis of plastid division in tomato fruit revealed that the majority of plastid division by binary fission occurs during the fruit enlargement stages when the plastids are present as chloroplasts. The plastid number remains fairly constant once ripening commences (Cookson et al., 2003). Replication of chromoplasts is occasionally observed, such as in pepper fruit (Leech and Pyke 1988) and *Forsythia suspensa* petals (Sitte 1987). Replication by budding and fragmentation has also been observed in the *suffulta* mutants in which a heterogeneous population of plastids exist (Forth and Pyke 2006). In agreement with the absence or low rate of division in regular tomato chromoplasts, only few members of the plastid division machinery have been encountered in the proteome (Barsan et al., 2010). Several homologs of the 3 FtsZ proteins of Arabidopsis have been detected, but the other parts of the plastid division machinery (Pyke 2007) such as ACCUMULATION AND REPLICATION OF CHLOROPLASTS5 (ARC5), PLASTID DIVISION1 (PVD1) and PLASTID DIVISION2 (PVD2) were absent.

iii) Internal membrane remodeling during chromoplast formation

Electron microscopy studies carried out in red pepper by Spurr and Harris (1968) have shown that remodeling of the internal membrane system starts with the lysis of the grana and the intergranal thylakoids. Some small and loosely aggregated groups of initial thylakoids still persist at advanced stages of ripening. In parallel new membrane systems are formed consisting in organized membrane complexes named thylakoid plexus by Spurr and Harris (1968) and thylakoid sheets. These early observations are consistent with most recent data (Simkin et al., 2007) showing that during the chloroplast-chromoplast conversion in tomato fruit the thylakoid

disassembly is associated with the synthesis of new membranes that are the site for the formation of carotenoid crystals. These newly synthesized membranes do not derive from the thylakoids but rather from vesicles generated from the inner membrane of the plastid (Simkin et al., 2007).

The loss of thylakoid integrity revealed by ultrastructure studies corresponds to a late event of chromoplast development which is visible well after the loss of thylakoid-associated metabolic functions. For instance, in the flower bud of *Lilium longiflorum* the rapid decline of photosynthetic activity during the chloroplast-chromoplast transition occurs well before any observable loss of thylakoid integrity and reduction of chlorophyll (Clément et al., 1997). The metabolic machineries are not affected at the same rate. Within the photosynthetic apparatus, photosystem II integrity was preserved longer than the rest of the machinery (Juneau et al., 2002). Interestingly, the loss of thylakoid integrity during tomato fruit ripening is associated with a strong decrease of a thylakoid-associated DNA-binding protein, MFP1, which is supposed to participate in the development of the thylakoid membrane (Jeong et al., 2003).

iv) Role of plastoglobules and plastoglobulins in the storage of carotenoids

During the chloroplast-chromoplast transition an increase in size and number of plastoglobuli is generally observed (Harris and Spur 1969). Microscopy studies demonstrated that plastoglobules arise from a blistering of the stroma-side leaflet of the thylakoid membrane predominantly along highly curved margins (Austin et al., 2006).

There is experimental evidence that plastoglobulins participate in the sequestration of carotenoids and in the biogenesis of chromoplasts (reviewed by Bréhélin and Kessler 2008). The observation that the suppression in tomato plants of the plastoglobulin carotenoid-associated protein (CHRC) results in 30% reduction of carotenoids in tomato flowers provided the first evidence for the role plastoglobulins chromoplast differentiation (Leitner-Dagan et al., 2006). Another evidence was given by the over-expression in tomato of a pepper plastoglobulin, fibrillin, which caused an increase in carotenoid and carotenoid-derived flavor volatiles (Simkin et al., 2007). In addition, the loss of thylakoids was delayed during the chloroplast to chromoplast transition and the plastids showed a typical chromoplastic zone contiguous with a preserved chloroplastic zone. It is concluded that fibrillin plays a role in thylakoid disorganization during chromoplast formation.

Plastoglobules not only act as lipid storage bodies, but they also participate in some metabolic pathways (Bréhélin and Kessler 2008). Analysis of the proteome of red pepper plastoglobules indicated the presence of several proteins involved in the synthesis of carotenoids, including ζ-carotene desaturase, lycopene β-cyclase and two β-carotene β-hydroxylases (Ytterberg et al., 2006). The ζ-carotene desaturase has been detected in the proteome of tomato chromoplasts (Barsan et al., 2010).

A scheme of the transition chloroplast-chromoplast is given in fig. 2 in which remodeling of internal membrane system and formation of carotenoid-storage structures are represented.

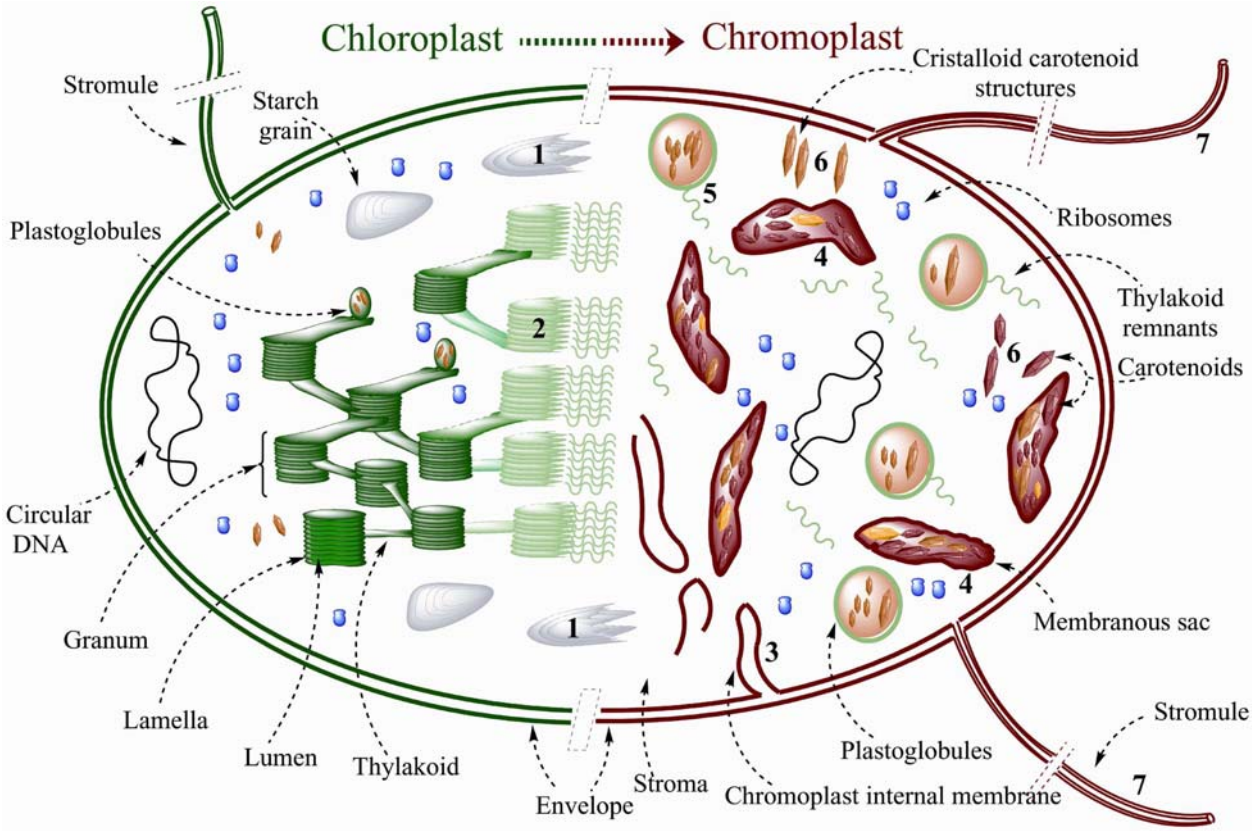


Figure 2. Schematic representation of the chloroplast-to-chromoplast transition. The scheme shows: the breakdown of starch granules (1) and of grana and thylakoids (2), the synthesis of new membrane structures form the inner membrane envelope of the plastid (3) leading to the formation carotenoid-rich membranous sacs (4), the increase in number and size of plastoglobules (5) the appearance of carotenoid-containing crystalloids (6) and the increase in the number protrusions emanating from the plastid envelope, named stromules (7).

v) Changes in stromules morphology during chromoplastogenesis

Stromules are motile protrusions emanating from the plastid membrane into the cytoplasm. Microscopy techniques coupled with GFP have revealed that the importance of stromules generally increases with the progress of fruit ripening in tomato (Waters et al., 2004). However there are some differences between tomato tissues. Long stromules are associated with plastids that are further apart, whereas short stromules are present in cells with a high density of plastids. In the outer mezocarp where cells have a high density of plastids, stromules are short and form a complex chromoplast network (Pyke and Howells 2002). In the inner mezocarp, the density of plastids is lower and stromules are longer and their number and length increases during ripening (Waters et al., 2004). Once the fruit begins to ripen, stromules increase in number and length, at least in the inner mesocarp, probably for providing greater import area for novel proteins (Kwok and Hanson 2004) particularly those involved in carotenoid biosynthesis and chromoplast differentiation. Sometimes, free broken stromules detached from the plastid appear as small vesicles containing only GFP throughout the cytoplasm of green fruit. They may have the potential to develop into full chromoplasts (Waters et al., 2004). The *green flesh* mutation in which plastid differentiation is incomplete and the *rin* mutation in which the ripening process is blocked result in a reduction of stromule formation (Waters et al., 2004).

3. Characteristics and stability of the chromoplastic genome during differentiation into chromoplasts

The plastid genome (plastome) of the tomato fruit has the same basic characteristics than the majority of the plastomes that have been sequenced. The size of the tomato fruit plastome is 155,461-bp and comprises a large and a small single copy region intercalated by two inverted repeats, IR_a and IR_b (Kahlau et al., 2006). Annotation indicated the presence of 114 genes and conserved open reading frames (ORFs) divided in three major categories (Sugiura 1992): i) photosystem- related genes, ii) genetic system genes, including genes encoding ribosomal proteins, tRNA and a plastid RNA polymerase and iii) the hypothetical chloroplast reading frames (*ycfs*), a group of conserved sequences, some of them of unknown function, but essential for plastids activity (Ravi et al., 2008).

Comparison by restriction enzyme analysis of DNA of chloroplasts of leaves and chromoplasts of tomato fruit revealed the absence of re-arrangements, losses or gains (Hunt et al.,

1986). However subtle changes in DNA, such as increased methylation of cytosine have been suggested upon analysis by liquid chromatography of tomato chromoplast (Kobayashi et al., 1990). However this observation was not confirmed by Marano and Carrillo (1991) who found that the patterns of DNA methylation assessed after restriction and hybridization analysis with DNA probes did not differ significantly between chloroplasts of mature green tomatoes and chromoplasts of red ripe fruit. Since structural and methylation changes in DNA have not been firmly established, their role in plastid switching remains uncertain.

4. Importance of transcriptional and translational activity during chromoplast differentiation

The expression pattern of few plastid localized genes has been studied. As expected, genes involved in carotenoid biosynthesis such as the *LYCOPENE β-CYCLASE* (*CYCB*) are up-regulated during chromoplast formation in many plants including citrus fruit (Alquezar et al., 2009), the wild species of tomato *Solanum habrochaites* (Dalal et al., 2010), safron (Ahrazem et al., 2010), papaya fruit (Blas et al., 2010) and carrot (Chen et al., 2001). On the contrary genes involved in photosynthetic activity are generally down-regulated during chromoplast formation (Cheung et al., 1993). Surprisingly, up-regulation of the large subunit of *RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE/OXYGENASE* and the 32 kD *PHOTOSYSTEM II QUINONE BINDING PROTEIN* genes has been observed in chromoplasts of squash fruits (*Cucurbitae pepo*) (Obukosia et al., 2003), indicating that the expression pattern of these photosystem genes could be regulated independently from the plastid differentiation processes.

A comprehensive study of chromoplastic transcriptome has been carried out by Kahlau and Bock (2008) showing that the global transcriptional activity remains almost the same during chromoplast differentiation, except for a limited number of genes, including (i) *accD*, which encodes a subunit of the acetyl-CoA carboxylase involved in fatty acid biosynthesis, (ii) the *trnA* (tRNA-ALA) and (iii) *rpoC2* gene (RNA polymerase subunit). During fruit ripening a reduction of translational activity has been observed by comparison of polysome-associated plastidial mRNAs levels between fruit chloroplasts and chromoplasts. Rather than a decrease in transcription, plastid translation appears to be the main factor that contributes to down-regulation of chromoplast proteins during fruit plastid differentiation. Another line of evidence to support this hypothesis is that the activity of both the nuclear encoded and plastid encoded RNA polymerases undergo little changes during the transition. Likewise, RNA splicing activity of the

plastid a possible mechanism contributing to the regulation of gene expression, exhibit not significant changes during tomato fruit ripening. In any case, transcriptional and translational activities of the plastid bring a limited contribution to chromoplast differentiation. The large majority of the proteins present in the plastid are encoded by nuclear genes so that transcriptional activity in the nucleus and translocation of proteins into the plastid are of primary importance for the build-up of the chromoplast metabolism. Proteins related to the biosynthesis of fatty acids, amino acids, carotenoids, vitamins, hormones, aroma volatiles and others have been encountered in the chromopalstic proteome of tomato (Barsan et al., 2010). These proteins participate in giving the fruit important sensorial characteristics such as color and aromas. Many of the corresponding genes are regulated by the plant hormone ethylene and therefore participate in the transcriptional regulation of the fruit ripening process in general (Giovannoni 2001, Pirrello et al., 2010). As shown in the following paragraph, some of the nuclear-localized genes play a crucial role in chromoplast differentiation.

5. Genes involved in chromoplast differentiation and development of carotenoid storage structures

Due to increased expression during the choloroplast to chromoplast transition some genes have been suspected to play a role in the chromoplastogenesis. Such is the case for the *EARLY LIGHT-INDUCIBLE PROTEIN* (*ELIP*) gene which has homology with light-harvesting complex proteins and whose expression is high during the breaker/turning ripening stages in tomato. However no direct evidence for a role of *ELIP* or other genes in chromoplast differentiation has been provided until the discovery of the cauliflower *Or* gene (Lu et al., 2006). The dominant mutation *Or* confers an orange pigmentation with accumulation of β-carotene mostly in the inflorescence of cauliflower without significantly affecting the expression of carotenoid biosynthetic genes (Li et al., 2001). Chromoplasts differentiate in the *OR* mutant and develop membranous inclusions of carotenoids resembling those of carrot roots. In addition, there was an arrest in plastid division and for this reason only one or two chromoplast are present in the affected cell (Paolillo et al., 2004). Chromoplast differentiation occurs mostly in the inflorescence tissues but not in the leaves, suggesting that tissue-specific expression is regulated at the transcriptional or posttranscriptional levels. The *Or* transgene introduced in a tuber-specific manner into potato induces a sharp increase in the accumulation of carotenoids again without affecting the

expression of endogenous carotenoid biosynthetic genes (Lu et al., 2006; Lopez et al., 2008). The *Or* gene is nuclear-localized and encodes a DnaJ-like co-chaperone containing a Cysteine-rich domain lacking the J-domain (Lu et al., 2006). The role of the DnaJ proteins is to interact with Hsp70 chaperones to perform protein folding, assembly, disassembly and translocation. The absence of phenotype upon RNAi silencing suggests that *Or* is not a loss of function mutation and putative interaction with Hsp70 chaperones indicates that it might be a dominant-negative mutation (Giuliano and Diretto 2007). Altogether, these data show that the *Or* gene is not directly involved in carotenoid biosynthesis but rather causes a metabolic sink for carotenoid accumulation through inducing the formation of chromoplasts (Li and van Eck 2007).

The role of some genes in the formation of carotenoid-storage structures has been explored. Over-expression of phytoene synthase gene causes carotenoid crystal formation on non-green tissues of Arabidopsis, but not in green tissues indicating fundamental difference in carotenoid storage mechanisms (Maass et al., 2009). Therefore the sequestration of carotenoids into crystals resulting from high activity of phytoene synthase can happen in the absence of chromoplast developmental programme such as in Arabidopsis, as a consequence of enhanced carbon flux through the pathway. High phytoene synthase has also associated with β-carotene accumulation in orange carrot roots (Maass et al., 2009).

6. Metabolic activities of chromoplasts

The metabolic activity of chromoplasts has been already reviewed (Neuhaus and Emes 2000, Bouvier and Camara 2007). We only give here a synthetic overview of the main features and include some recent data. When chromoplasts derive from chloroplasts, the most obvious biochemical change is the loss of chlorophyll and photosynthetic activity associated with the down-regulation of photosynthetic gene expression (Piechulla et al., 1985). Another major feature of chromoplasts metabolism is the accumulation of pigments. Several reviews have been dedicated to the biosynthesis of carotenoids in fruit and flowers (Bramley 2002, Fraser and Bramley 2004, Lu and Li 2008). However, they are also the site for synthesis of sugars, starch, lipids, aromatic compounds, vitamins (riboflavine, folate, tocopherols) and hormones (Neuhaus and Emes 2000, Barsan et al., 2010). For sustaining biosynthetic activities, sugars are imported from the cytosol by a plastid-localized glucose transporter (Bouvier and Camara 2007) but the use of endogenous sugars resulting from starch degradation cannot be excluded. Proteins of

starch biosynthesis and degradation remain present in tomato chromoplasts (Barsan et al., 2010). Calvin cycle enzymes have been measured in plastids isolated from sweet pepper and their activities were generally greater in chromoplasts than in chloroplasts (Thom et al., 1998). In tomato, activity of enzymes of the Calvin Cycle has also been observed (Obiadalla-Ali et al., 2004). In association with the persistence of active oxidative phosphate pathway (Tetlow et al., 2003, Bouvier and Camara 2007, Barsan et al., 2010) ATP and reducing power are produced that also participate in sustaining the metabolic activities of chromoplasts. Another interesting feature of the chromoplast is the presence of highly active antioxidant system. The level of glutathione and ascorbate in the plastids isolated from pepper fruit increase during fruit ripening in parallel with the activity of the enzymes of the ascorbate glutathione cycle and superoxide dismutase (Marti et al., 2009). High activity of the antioxidant system in the chromoplast could play a role in protecting plastid components such as carotenoids against oxidation, but also in mediating signaling between chromoplast and nucleus. Reactive oxygen species are considered as participating in the plastid to nucleus communication (Kleine et al., 2009, Galvez-Valdivieso and Mullineaux 2010). Plastid generated reactive oxygen species are known to up-regulate the transcription of genes of carotenoid biosynthesis (Bouvier et al., 1998).

7. Reversible differentiation of chromoplasts

Reversible differentiation of plastids is another aspect of plasticity of the organelle. Preberg et al., (2008) quote a number of situations where re-greening of tissues occurs as a consequence of re-differentiation of gerontoplasts, etioplasts or chromoplasts into chloroplasts. The phenomenon is truly a re-differentiation process without any evidence of *de novo* generation of plastids or plastid division. In the case of chromoplasts, the best known example of reversal to chloroplasts is that of citrus fruits (Thomson et al., 1967) but the phenomenon also exist in other species such in cucurbits fruit (Preberg et al., 2008). Ultrastructural aspects of the reversion of chromoplasts to chloroplasts have been described in the sub-epidermal layer of fruit of *Cucurbita pepo* (Devide and Ljubesic 1974). During re-greening, the globular type chromoplasts with numerous plastoglobules and small vesicle-like fragments of thylakoids undergo a disappearance of plastoglobules and the formation of new thylakoids. Thylakoids arise from both pre-existing vesicles and from the invagination of the inner membrane of the plastid to form grana structures leading to normal chloroplast structure and photosynthetic activity. Similar reconstitution of the

thylakoid system has been described recently with more details during re-differentiation of chloroplasts in cucumber fruit (Preberg et al., 2008). In this case the plastoglobules persisted during the whole process and remnants of the degraded thylakoid system formed large membrane-bound bodies that later participated in the re-formation of thylakoids.

Light is probably the most important factor of re-greening via phytochromes, however nutritional factors are also involved. Warm temperatures, nitrogen fertilization and gibberellins stimulate re-greening of citrus peel, while abundance of sucrose tends to inhibit this process (Huff, 1983). Very little information is available on the molecular mechanisms of reversal from chromoplasts to chloroplasts. However, there is evidence that gibberellic acid which stimulates the greening process reduces the expression of carotenoid biosynthetic genes, phytoene synthase, phytoene desaturase and β-carotene hydroxylase in orange flavedo (Rodrigo and Zacarias 2007). In clementines, gibberellins and nitrate that favor re-greening reduced the expression of not only phytoene synthase, but also of the chlorophyll-degrading gene pheophorbide a oxygenase (Alos et al., 2006) indicating that re-greening involves both repression of carotenoid biosynthesis and reduction of chlorophyll breakdown. No information is available yet on the expression of genes involved in the biosynthesis of photosystems and chlorophyll.

8. Mutants with altered chromoplast development

We have already mentioned the *Or* mutant of cauliflower which has allowed the isolation of a gene controlling the differentiation of chromoplasts. Here we briefly examine other mutants, mostly of tomato, showing, among other phenotypes altered plastid development. Despite the pleiotropic effects of the mutation, these mutants represent useful tools for the identification of the molecular players involved in chromoplast biogenesis.

Compared to wild type, the natural mutants *HIGH PIGMENT 1* and *2* (*hp1* and *hp2*) have dark-green immature fruits and accumulate higher levels of carotenoids in ripe fruits (Yen et al., 1997, Mustilli et al., 1999). The *hp1* mutant codes a homologue of the Arabidopsis UV-DAMAGED DNA-BINDING PROTEIN 1 (DDB1) protein, which is predicted to interact with the nuclear factor DEETIOLATED 1 (DET1) (Liu et al., 2004), while the *hp2* codes a tomato orthologue of the *Det1* (Mustilli et al., 1999). Ripe fruits of both mutants contain more and bigger chromoplasts per cell than wild type fruits. The product of the *Det1* gene is part of the CUL4-based E3 ubiquitin ligase complex of the proteasome (Bernhardt et al., 2006, Wang et al., 2008).

In the *HIGH PIGMENT 3* mutant (*hp3*) which has the same phenotype as *hp1* and *hp2*, the level of abscisic acid (ABA) is lower than in wild type plants suggesting that ABA deficiency could be an important factor for the development of the phenotype. This hypothesis is further sustained by the analysis of two other ABA-deficient tomato mutants, *flacca* and *sitiens* that harbor similar alterations in plastid development (Galpaz et al., 2008).

The mutation in the locus *suffulta* provokes changes in the division of plastids in tomato plants, generating cells with giant chloroplasts but with low chlorophyll content. An unusual process of plastid division occurs during the chloroplast-to-chromoplast transition, characterized by budding and plastid fragmentation into small vesicles. This results in a heterogeneous population of chromoplasts at different development stages with some of them keeping the chloroplasts structure (Forth and Pyke 2006). The molecular identity of the gene responsible for *suffulta* phenotype is unknown. It could be a component of the plastidial division machinery or could regulate of the division process. It could also participate in the differentiation of the chromoplast.

In addition to light, phytohormones have been reported to play an important role in controlling chloroplast/chromoplast formation and stability during tomato fruit development. It is well known that tomato mutants and transgenic lines, impaired in elements of the ethylene signaling transduction cascade such as the ethylene receptor NR (Wilkinson et al., 1995) present altered pigmentation. Down-regulation of ARF4, an Auxin Response Factor formerly named DR12, resulted in dark-green phenotype and blotchy ripening of tomato fruit (Jones et al., 2002). In the ARF4 down-regulated lines, the outer pericarp tissue displayed a higher number of chloroplast per cell and a dramatic increase in grana formation. Interestingly, in contrast to *hp* mutants, the dark-green phenotype in ARF4-inhibited lines is confined to the fruit. The treatment of tomato fruits with fluridone, an inhibitor of ABA synthesis, has inhibitory effects on carotenoids accumulation (Zhang et al., 2009). It was also reported that exogenous treatment with cytokinin can mimic the *hp* mutant phenotype (Mustilli et al., 1999) and that *CYTOKININ-HYPERSENSITIVE* Arabidopsis mutants show increased chloroplast development (Kubo and Kakimoto 2000). The blotchy ripening phenotype was also induced by ectopic expression in tomato lines of the *ipt* gene from the Ti plasmid of *Agrobacterium tumefaciens* (Martineau et al., 1994). In this latter case, fruit displayed higher levels of cytokinin and during ripening the fruit exhibited altered phenotype with green patches remaining within a deep red background.

The *rin* tomato mutant harbors a non functional MADS-box transcription factor that is essential for fruit ripening (Vrebalov et al., 2002). The number of the chromoplasts per cell in *rin* fruits, at the breaker stage, is much higher than the wild type and the plastids are very small with few stromules. If the *RIN* gene is a direct regulator of the plastid transition in tomato fruit or the lack of the ethylene synthesis in *rin* fruits is the responsible for the abnormal chromoplast biogenesis it remains unclear.

The *green flesh* and the *chlorophyll retainer* are mutants of tomato and pepper, respectively, which has no ability to degrade chlorophyll during fruit ripening, but are able to synthesize carotenoids resulting in brown color fruits. The plastids in the ripe fruit of these two mutants have remnants of thylakoidal membranes and formation of plastoglobuli suggesting that the conversion of the chloroplasts to chromoplasts is not completely concluded. The level of the carotenoids in the mutants is lower than the wild type fruits and several photosystem genes, like *rbcL* and *cab* are up-regulated. Barry et al., (2008) have indicated the possibility that this mutation being due to an impaired gene product linked to the chlorophyll degradation pathway.

All mutations described above show evidence that the chromoplast formation is a complex event that involves not only factors expressed during the ripening, but also developmental factors and hormones like auxin, cytokinin, ABA and ethylene. Two processes seem to be important for normal chromoplast biogenesis, chloroplast division and the biosynthesis of the carotenoids. However, the way by which these processes are coordinated by nuclear and plastid gene expression remains unclear and represents a challenge for future studies.

9. Future perspectives

The functional genomics tools will allow new insights on the mechanisms of chromoplast development. As revealed by the comprehensive survey with the new mass spectrometry technologies, the number of proteins assigned to the chromoplast proteome (Barsan et al., 2010) is comparable to that of the chloroplast proteome (Ferro et al., 2010). The development of specific protocols for isolating plastids at different stages of differentiation associated with the use of comparative proteomic methods represent an interesting perspective towards the uncovering of target proteins that play a role in the chromoplast differentiation process.

Moreover, the combination of transcriptomic and proteomic data will allow identifying molecular events that are regulated at the transcriptional, posttranscriptional or posttranslational

levels. Particularly, the identification of phosphorylation and other types of protein modifications by proteomic analysis will give useful information on the levels of regulation of plastid metabolic activity during the chloro/chromoplast transition steps.

While a number of experimental data support the role of phythormones in regulating plastid differentiation and evolution during ripening, the underlying mechanisms remain unclear. Also, though many hormones like ethylene, auxin, cytokinin and ABA, seem to take part in the regulation of the chloroplast to chromoplast transition, the extent of the cross-talk between hormones to tune the process is unknown.

It is admitted that the expression of many genes targeted to plastids are regulated through a dialog between the nucleus and the plastid. These signals either environmental (temperature, light, etc) or developmental are supposed to comprise reactive oxygen species (ROS), carotenoids, carbohydrates and hormones (ABA, jasmonates). The presence of a plastid-nucleus dialog is testified by the fact that exposure of the chloroplast to tagetitoxin, a specific inhibitor of plastidial RNA polymerase (Rapp and Mullet 1991), or lincomycin, a specific inhibitor of plastid peptidyl transferase (Mulo et al., 2003), decreases the accumulation of plastid targeted nuclear transcripts. However, the contribution of these signals to the expression of specific genes is far from being fully understood (Kleine et al., 2009). In addition, most of the studies dealing with nucleus to plastid signaling have been carried out with chloroplasts. Whether some of these mechanisms are active in non-photosynthetic plastids, such as chromoplasts, remains an open question. In these conditions, we are still far from a clear understanding of the dialog between the nuclear and the plastidial genome in mediating the differentiation of the chromoplast and, beyond, in controlling developmental processes such as fruit ripening and flower development.

References

Ahrazem, O., Rubio-Moraga, A., Castillo López, R. and Gómez-Gómez, L. (2010) The expression of a chromoplast-specific lycopene beta cyclase gene is involved in the high production of saffron's apocarotenoid precursors. J. Exp. Bot. 61: 105-119.

Alos, E., Cercos, M., Rodrogo, M.J., Zacarias, L. and Talon, M. (2006) Regulation of color break in citrus fruit. Changes in pigment profiling and gene expression induced by gibberellins and nitrate, two ripening retardants. *J. Agr. Food Chem.* 54: 4888-4895.

Alquezar, B., Zacarías, L. and Rodrigo, M.J. (2010) Molecular and functional characterization of a novel chromoplast-specific lycopene β-cyclase from Citrus and its relation to lycopene accumulation. *J. Exp. Bot.* 60: 1783-1797.

Austin, J.R., Frost, E., Vidi, P., Kessler, F. and Staehelin, L.A. (2006) Plastoglobules are lipoproteins subcompartments of the chloroplast that are permanently coupled to thylakoid membranes and contain biosynthetic enzymes. *Plant Cell* 18: 1693-1703.

Baginsky, S., Siddique, A. and Gruissem, W. (2004) Proteome analysis of tobacco bright yellow-2 (BY-2) cell culture plastids as a model for undifferentiated heterotrophic plastids. *J. Proteome Res.* 3: 1128-1137.

Balmer, Y., Vensel, W.H., DuPont, F.M., Buchanan, B.B. and Hurkman, W.J. (2006) Proteome of amyloplasts isolated from developing wheat endosperm presents evidence of broad metabolic capability. *J. Exp. Bot.* 57: 1591-1602.

Barry, C.S., McQuinn, R.P., Chung, M.Y., Besuden, A. and Giovannoni, J.J. (2008) Amino acid substitutions in homologs of the STAY-GREEN protein are responsible for the *green-flesh* and *chlorophyll retainer* mutations of tomato and pepper. *Plant Physiol.* 147: 179-187.

Barsan, C., Sanchez-Bel, P., Rombaldi, C., Egea, I., Rossignol, M., Kuntz, M., Zouine, M., Latché, A., Bouzayen, M. and Pech, J.C. (2010). Characteristics of the tomato chromoplast proteome revealed by proteomic analysis. *J. Exp. Bot.* 61: 2413-2431.

Bathgate, B., Purton, M.E., Grierson, D. and Goodenough, P.W. (1985) Plastid changes during the conversion of chloroplasts to chromoplasts in ripening tomatoes. *Planta* 165: 197-204.

Ben-Shaul, Y. and Klein, S. (1965) Development and structure of carotene bodies in carrot roots. *Botan. Gaz.*126: 79-85.

Bernhardt, A., Lechner, E., Hano, P., Schade, V., Dieterle, M., Anders, M., Jody Dubin, M., Benvenuto, G., Bowler, C., Genschik, P. and Hellmann, H. (2006). CUL4 associates with DDB1 and DET1 and its downregulation affects diverse aspects of development in *Arabidopsis thaliana. Plant J.* 47:591-603.

Blas, A.L., Ming, R., Liu, Z., Veatch, O.J., Paull, R.E., Moore, P.H. and Yu, Q. (2010) Cloning of papaya chromoplast specific lycopene β-cyclase, *CpCYC-b*, controlling fruit flesh color reveals conserved microsynteny and a recombination hotspot. *Plant Physiol*. 152: 2013-2022.

Bonora, A., Pancaldi, S., Gualandri, R. and Fasulo, M.P. (2000). Carotenoid and ultrastructure variations in plastids of *Arum italicum* Miller fruit during maturation and ripening. *J. Exp. Bot.* 51: 873-884.

Bouvier, F., Backhaus, R.A. and Camara, B. (1998) Induction and control of chromoplast-specific carotenoid genes by oxidative stress. *J. Biol. Chem.* 273: 30651-30659.

Bouvier, F. and Camara, B. (2007) The role of plastids in ripening fruits. In *The structure and functions of plastids*. Edited by Wise, R.R. and Hoober, J.K. pp. 419-432. Springer, Dordrecht.

Bramley, P.M. (2002) Regulation of carotenoid formation during tomato fruit formation and development. *J. Exp. Bot.* 53: 2107-2113.

Bravdo, B., Palgi, A. and Lurie, S. (1977) Changing ribulose diphosphate carboxylase/oxygenase activity in ripening tomato fruit. *Plant Physiol.* 60: 309-312.

Bréhélin, C. and Kessler, F. (2008) The plastoglobule: a bag full of lipid biochemistry tricks. *Photochem. Photobiol.* 84: 1388-1394.

Büker, M., Schünemann, D. and Borchert, S. (1998) Enzymic properties and capacities of developing tomato (*Lycopersicon esculentum* L.) fruit plastids. *J. Exp. Bot.* 49: 681-691.

Camara, B., Hugueney, P., Bouvier, F., Kuntz, M. and Monéger, R. (1995) Biochemistry and molecular biology of chromoplast development. *Int. Rev. Cytol.* 163: 175–247

Chen, D.M., Xue, Y., Liu, J.M., Wang, Y.J. and Chen, H. (2001) Isolation of lycopene beta-cyclase cDNA from *Daucus carota* and its differential expression in roots. *Acta. Bot. Sinica.* 43: 1265-1270

Cheung, A.Y., McNellis, T. and Piekos, B. (1993) Maintenance of chloroplast components during chromoplast differentiation in the tomato mutant green flesh. *Plant Physiol.* 101: 1223-1229.

Clément, C., Mischler, P., Burrus, M. and Audran, J.C. (1997) Characteristics of the photosynthetic apparatus and CO_2 fixation in the flower bud of *Lilium I.* corolla. *Int. J. Plant Sci.* 158: 794-800.

Cookson, P.J., Kiano, J., Fraser, P.D., Romer, S., Shipton, C.A., Schuch, W., Bramley, P.M. and Pyke, K.A. (2003) Increases in cell elongation, plastid compartment size and translational control of carotenoid gene expression underly the phenotype of the *high pigment-1* mutant of tomato. *Planta* 217: 896-903.

Dalal, M., Chinnusamy, V. and Bansal, K.C. (2010) Isolation and functional characterization of *Lycopene β-cyclase* (*CYC-B*) promoter from *Solanum habrochaites. BMC Plant Biol*. 10: 61-76.

Deruere, J., Romer, S., d'Harlingue, A., Backhaus, R.A., Kuntz, M. and Camara, B. (1994) Fibril assembly and carotenoid over-accumulation in chromoplasts: a model for supramolecular lipoprotein structures. *Plant Cell* 6: 119–133.

Devidé, Z. and Ljubesic, N. (1974) The reversion of chromoplasts to chloroplasts in pumpkin fruits. *Z. Planzenphysiol.* 73: 296-306.

Ferro, M., Brugière, S., Salvi, D., Seigneurin-Berny, D., Court, M., Moyet, L., Ramus, C., Miras, S., Mellal, M., Le Gall, S., Kieffer-Jaquinod, S., Bruley, C., Garin, J., Joyard, J., Masselon, C. and Rolland, N. (2010). AT_CHLORO, a comprehensive chloroplast proteome database with subplastidial localization and curated information on envelope proteins. *Mol. Cell. Proteomics* 9: 1063-1084.

Forth, D. and Pyke, K.A; (2006) The *suffulta* mutation in tomato reveals a novel method of plastid replication during fruit ripening. *J. Exp. Bot.* 57: 1971-1979.

Fraser, P.D. and Bramley, P.M. (2004) The biosynthesis and nutritional uses of carotenoids. *Prog. Lipid Res.* 43: 228–265.

Galpaz, N., Wang, Q., Menda, N., Zamir, D. and Hirschberg, J. (2008) Abscisic acid deficiency in the tomato mutant high-pigment 3 leading to increased plastid number and higher fruit lycopene content. *Plant J.* 53: 717-730.

Galvez-Valdivieso, G. and Mullineaux, P.M. (2010) The role of reactive oxygen species in signalling from chloroplasts to the nucleus. *Physiol. Plant.* 138: 430–439

Giovannoni, J.J. (2001) Molecular regulation of fruit ripening. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52: 725-749.

Giuliano, G. and Diretto, G. (2007) Of chromoplasts and chaperones. *Trends Plant Sci.* 12: 529-531.

Grilli-Caiola, M.G. and Canini, A. (2004) Ultrastructure of chromoplasts and other plastids in *Crocus sativus* L. (Iridaceae). *Plant Biosystems* 138: 43-52.

Harris, W.M. and Spurr, A.R. (1969) Chromoplasts of tomato fruit. II. The red tomato. *Amer. J. Bot.* 56: 380-389.

Horner, H.T., Healy, R.A., Ren, G., Fritz, D., Klyne, A., Seames, C. and Thronburg, R.W. (2007) Amyloplast to chromoplast conversion in developing ornamental tobacco floral nectaries provides sugar for nectar and antioxidants for protection. *Amer. J. Bot*. 94, 12-24.

Huff, A. (1983) Nutritional control of regreening and degreening in citrus peel segments. *Plant Physiol.* 73: 243-249.

Hunt, C.M., Hardison, R.C. and Boyer, C.D. (1986) Restriction enzyme analysis of tomato chloroplast and chromoplast DNA. *Plant Physiol*. 82: 1145-1147.

Jeong, S.Y., Rose, A. and Meier, I. (2003) MFP1 is a thylakoid-associated, nucleoid-binding protein with a coiled-coil structure. *Nucleic Acid Res*. 31: 5175-5185.

Jones, B., Frasse, P., Olmos, E., Zegzouti, H., Li, Z.G., Latché, A., Pech, J.C. and Bouzayen, M. (2002) Down-regulation of DR12, an auxin-response-factor homolog, in the tomato results in a pleiotropic phenotype including dark green and blotchy ripening fruit. *Plant J*. 32: 603-613.

Juneau, P., Le Lay, P., Böddi, B., Samson, G. and Popovic, R. (2002) Relationship between the structural and functional changes of the photosynthetic apparatus during chloroplast-chromoplast transition in flower bud of *Lilium longiflorum*. *Photochem. Photobiol.* 75: 377-381.

Kahlau, S., Aspinall, S., Gray, J.C. and Bock, R. (2006) Sequence of the tomato chloroplast DNA and evolutionary comparison of Solanaceous plastid genomes. *J. Mol. Evol*. 63: 194-207.

Kahlau, S. and Bock, R. (2008) Plastid transcriptomics and translatomics of tomato fruit development and chloroplast-to-chromoplast differentiation: Chromoplast gene expression largely serves the production of a single protein. *Plant Cell* 20: 856-874.

Kim, J.E., Rensing, K.H., Douglas, C.J. and Cheng, K.M. (2010) Chromoplast ultrastructure and estimated carotene content in root secondary phloem of different carrot varieties. *Planta* 231: 549-558.

Kleine, T., Voigt, C. and Leister, D. (2009) Plastid signalling to the nucleus: messengers still lost in the mists? *Trends Gen.* 25: 185-190.

Kobayashi, H., Ngernprasirtsiri, J. and Akazawa, T. (1990) Transcriptional regulation and DNA methylation in plastids during transitional conversion of chloroplasts to chromoplasts. *EMBO J.* 9: 307-313.

Köhler, R.H. and Hanson, M.R. (2000) Plastid tubules of higher plants are tissue-specific and developmentally regulated. *Cell Sci.* 113: 81-89.

Kubo, M. and Kakimoto, T. (2000) The *CYTOKININ-HYPERSENSITIVE* genes of Arabidopsis negatively regulate the cytokinin-signaling pathway for cell division and chloroplast development. *Plant J.* 23: 385-394.

Kwok, E.Y. and Hanson, M.R. (2004) GFP-labelled Rubisco and aspartate aminotransferase are present in plastid stromules and traffic between plastids. *J. Exp. Bot.* 55: 595-604.

Laborde, J.A. and Spurr, A.R. (1973) Chromoplast ultrastructure as affected by genes controlling grana retention and carotenoids in fruits of *Capsicum annuum*. *Amer. J. Bot.* 60: 736-744.

Leech, R.M. and Pyke, K.A. (1988) Chloroplast division in higher plants with particular reference to wheat. In *The Division and Segregation of Organelles* Edited by Boffey, S.A. and Lloyd, D. pp. 39-61. Cambridge University Press, Cambridge, UK.

Leitner-Dagan, Y., Ovadis, M., Shklarman, E., Elad, Y., David, D.R. and Vainstein, A. (2006) Expression and functional analysis of the plastid lipid-associated protein CHRC suggest its role in chromoplastogenesis and stress. *Plant Physiol*. 142: 233-244.

Li, L., Paolillo, D.J., Parthasarathy, M.V., DiMuzio, E.M. and Garvin, D.F. (2001) A novel gene mutation that confers abnormal patterns of β-carotene accumulation in cauliflower (*Brassica oleracea* var. *botrytis*). *Plant J.* 26: 59-67.

Li, L. and van Eck, J. (2007) Metabolic engineering of carotenoid accumulation by creating a metabolic sink. *Transg. Res.* 16: 581-585.

Liu, Y., Roof, S., Ye, Z., Barry, C., van Tuinen, A., Vrebalov, J., Bowler, C. and Giovannoni, J.J. (2004) Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato. *Proc. Natl Acad. Sci. USA* 101: 9897–9902.

Ljubesic, N., Wrischer, M. and Devise, Z. (1991) Chromoplasts-the last stages in plastid development. *Int. J. Dev. Biol.* 35: 251-258.

Ljubesic, N., Wrischer, M. and Device, Z. (1996) Chromoplast structures in *Thunbergia* flowers. *Protoplasma* 193: 174-180.

Lopez, A.B., van Eck, J.V., Conlin, B.J., Paolillo, D.J., O'Neill, J. and Li, L. (2008). Effect of the cauliflower *Or* transgene on carotenoid accumulation and chromoplast formation in transgenic potato tubers. *J. Exp. Bot.* 59: 213-223.

Lopez-Juez, E. (2007) Plastid biogenesis, between light and shadows. *J. Exp. Bot.* 58:11-26.

Lopez-Juez, E. and Pyke, K.A. (2005) Plastids unleashed: their development and their integration in plant development. *Int. J. Dev. Biol.* 49: 557-577

Lu, S. and Li, L. (2008) Carotenoid metabolism: Biosynthesis, regulation,and beyond. *J. Integr. Plant Biol.* 50: 778–785.

Lu, S., van Eck, J., Zhou, X., Lopez, A.B., O'Halloran, D.M., Cosman, K.M., Conlin, B.J., Paolillo, D.J., Garvin, D.F., Vrebalov, J., Kochian, L.V., Küpper, H., Earle, E.D., Cao, J. and Li, L. (2006) The cauliflower *Or* Gene encodes a DnaJ cysteine-rich domain-containing protein that mediates high levels of ß-carotene accumulation. *Plant Cell* 18: 3594-3605.

Maass, D., Arango, J., Wüst, F., Beyer, P. and Welsh, R. (2009) Carotenoid crystal formation in Arabidopsis and carrot roots caused by increased phytoene synthase protein levels. *PloS ONE* 4: 6373.

Marano, M.R. and Carrillo, N. (1991) Chromoplast formation during tomato fruit ripening - no evidence for plastid DNA methylation. *Plant Mol. Biol.* 16: 11-19.

Marano, M.R., Serra, E.C., Orellano, G. and Carrillo, N. (1993). The path of chromoplast development in fruits and flowers. *Plant Sci.* 94: 1-17.

Marti, M.C., Camejo. D., Olmos, E., Sandalio, L.M., Fernandez-Garcia, N., Jimenez, A. and Sevilla, F. (2009) Characterisation and changes in the antioxidant system of chloroplasts and chromoplasts isolated from green and mature pepper fruits. *Plant Biosystems* 11: 613-624.

Matile, P. (2000) Biochemistry of Indian summer: physiology of autumnal leaf coloration. *Exp. Gerontol.* 35: 145-158.

Martineau, B., Houck, C.M., Sheehy, E. and Hiatt, W.R. (1994) Fruit-specific expression of the *A. tumefaciens* isopentenyl transferase gene in tomato: effects on fruit ripening and defense-related gene expression in leaves. *Plant J.* 5: 11-19.

Mulo, P., Pursiheimo, S., Hou, C.X., Tyystjarvi, T. and Aro, E.M. (2003) Multiple effects of antibiotics on chloroplast and nuclear gene expression. *Funct. Plant Biol*. 30: 1097-1103

Mustilli, A.C., Fenzi, F., Ciliento, R., Alfano, F. and Bowler, C. (1999). Phenotype of the tomato high pigment-2 mutant is caused by a mutation in the tomato homolog of DEETIOLATED1. *Plant Cell* 11: 145-157

Neuhaus, H.E. and Emes, M.J. (2000) Non-photosynthetic metabolism in plastids. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 51: 111-140.

Obiadalla-Ali, H., Fernie, A.R., Kossmann, J. and Lloyd, J.R. (2004) Developmental analysis of carbohydrate metabolism in tomato (*Lycopersicon esculentum* cv. Micro-Tom) fruits. *Physiol. Plant.* 120: 196–204

Obukosia, S.D., Richards, C.M. and Boyer, C.D. (2003) Expression of plastid-encoded photosynthetic genes during chloroplast or chromoplast differentiation in *Cucurbitae pepo* L. fruits. *Phytochemistry* 64: 1213-1221, 2003.

Paolillo, D.J., Garvin, D.F. and Parthasarathy, M.V. (2004) The Chromoplasts of Or mutants of cauliflower (Brassica oleracea L. var. botrytis). *Protoplasma* 224: 245-253.

Piechulla, B., Chonoles Imlay, K.R. and Gruissem, W. (1985) Plastid gene expression during fruit ripening in tomato. *Plant Mol. Biol.* 5: 373-385.

Pirrello, J., Regad, F., Latché, A., Pech, J.C. and Bouzayen, M. (2009) Regulation of Tomato Fruit Ripening. In *Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*. *CAB Reviews* 4: 1-14

Preberg, T., Wrisher, M., Fulgosi, H. and Ljubesic, N. (2008) Ultrastructural characterization of the reversible differentiation of chloroplasts in cucumber fruit. *J. Plant Physiol*. 51: 122-131.

Pyke, K.A. (2007) Plastid biogenesis and differentiation. In *Topics in Current Genetics, Cell and molecular biology of plastids.* Edited by Bock, R. 19:1-28. Springer-Verlag, Berlin, Heidelberg.

Pyke, K.A. and Howells, C.A. (2002) Plastid and stromule morphogenesis in tomato. *Ann. Bot.* 90: 559-566.

Rapp, J.C. and Mullet, J.E. (1991) Chloroplast transcription is required to express the nuclear genes *rbcs* and *cab* plastid DNA copy number is regulated independently. *Plant Mol. Biol.* 17: 813-823.

Ravi, V., Khurana, J.P., Tyagi, A.K. and Khurana, P. (2008) An update on chloroplast genomes. *Plant Syst. Evol*. 271: 101-122.

Rodrigo, M.J. and Zacarias, L. (2007) Effect of postharvest ethylene treatment on carotenoid accumulation and the expression of carotenoid biosynthetic genes in the flavedo of orange (*Citrus sinensis* L. Osbeck) fruit. *Postharvest Biol. Technol*. 43: 14-22

Siddique, M.A., Grossmann, J., Gruissem, W. and Baginsky, S. (2006) Proteome analysis of bell pepper (*Capsicum annuum* L.) chromoplasts. *Plant Cell Physiol*. 47: 1663-1673.

Simkin, A.J., Gaffé, J., Alcaraz, J.P., Carde, J.P., Bramley, P.M., Fraser, P.D. and Kuntz, M. (2007) Fibrillin influence on plastid ultrastructure and pigment content in tomato fruit. *Phytochemistry* 68:1545-1556.

Sitte, P. (1987) Development and division of chromoplasts in petals of *Forsythia*. *Cellule* 74: 59-77.

Spurr, A.R. and Harris, W.M. (1968) Ultrastructure of chloroplasts and chromoplasts in *Capsicum annuum*. I. Thylakoid membrane changes during fruit ripening. *Amer. J. Bot.* 55: 1210-1224.

Steinhauser, M.C., Steinhauser, D., Koehl, K., Carrari, F., Gibon, Y., Fernie, A.R. and Stitt, M. (2010) Enzyme activity profiles during fruit development in tomato cultivars and *Solanum pennellii*. *Plant Physiol*. 153: 80–98

Sugiura, M. (1992) The chloroplast genome. *Plant Mol. Biol.* 19: 149-168.

Tetlow, I.J., Bowsher, C.G. and Emes, M.J. (2003) Biochemical properties and enzymic capacities of chromoplasts isolated from wild buttercup (*Ranunculus acris* L.). *Plant Sci.* 165: 383-394.

Thom, E., Mohlmann, T., Quick, P.W., Camara, B. and Neuhaus, H.E. (1998) Sweet pepper plastids: enzymic equipment, characterisation of the plastidic pentose-phosphate pathway, and transport of phosphorylated intermediates across the envelope membrane. *Planta* 204: 226–233.

Thomson, W.W., Lewis, L.N. and Coggins, C.W. (1967) The reversion of chromoplasts to chloroplasts in Valencia oranges. *Cytologia* 32: 117-124.

Thomson, W.W. and Whatley, J.M. (1980) Development of nongreen plastids. *Ann. Rev. Plant Physiol.* 31: 375-394.

Vasquez-Caicedo, A.L., Heller, A., Neidhart, S. and Carle, R. (2006) Chromoplast morphology and β-carotene accumulation during postharvest ripening of Mango Cv. “Tommy Atkins”. *J. Agr. Food Chem.* 54: 5769-5776.

Vidi, P.A., Kanwischer, M., Baginsky, S., Austin, J.R., Csucs, G., Dormann, P., Kessler, F. and Bréhélin, C. (2006) Tocopherol cyclase (VTE1) localization and vitamin E accumulation in chloroplasts plastoglobule protein particles. *J. Biol. Chem.* 281: 11225-11234.

von Zychlinski, A., Kleffmann, T., Krishnamurthy, N., Sjölander, K., Baginsky, S. and Gruissem, W. (2005) Proteome analysis of the rice etioplast: metabolic and regulatory networks and novel protein functions. *Mol. Cell. Prot.* 4: 1072-1084.

Vrebalov, J., Ruezinsky, D., Padmanabhan, V., White, R., Medrano, D., Drake, R., Schuch, W. and Giovannoni, J. (2002) A MADS-box gene necessary for fruit ripening at the tomato Ripening-inhibitor (Rin) locus. *Science* 296: 343–346.

Wang, S., Liu, J., Feng, Y., Niu, X., Giovannoni, J. and Liu, Y. (2008) Altered plastid levels and potential for improved fruit nutrient content by down-regulation of the tomato DDB1-interacting protein CUL4. *Plant J.* 55: 89–103.

Waters, M.T. and Pyke, K.A. (2004) Plastid development and differentiation. In *Plastids*: Edited by Møller, S.G., pp. 30-59. Blackwell, Oxford, UK.

Waters, M.T., Fray, R.G. and Pyke, K.A. (2004) Stromule formation is dependent upon plastid size, plastid differentiation status and the density of plastids within the cell. *Plant J.* 39: 655-667.

Whatley, J.M. (1978) A suggested cycle of plastid development interrelationships. *New Phytologist* 80: 489-502.

Wilkinson, J.Q., Lanahan, M.B., Yen, H.C., Giovannoni, J.J. and Klee, H.J. (1995) An ethylene-inducible component of signal-transduction encoded by NEVER-RIPE. *Science* 270: 1807-1809.

Yen, H., Shelton, A., Howard, L., Vrebalov, J. and Giovannoni, J.J. (1997) The tomato *high pigment* (*hp*) locus maps to chromosome 2 and influences plastome copy number and fruit quality. *Theor. Appl. Genet.* 95: 1069-1079.

Ytterberg, A.J., Peltier, J.B. and van Wijk, K.J. (2006). Protein profiling of plastoglobules in chloroplasts and chromoplasts. A surprising site for differential accumulation of metabolic enzymes. *Plant Physiol.* 140: 984-997.

Zhang, M., Yuan, B. and Leng, P. (2009) The role of ABA in triggering ethylene biosynthesis and ripening of tomato fruit. *J. Exp. Bot*. 60: 1579-1588.

CHAPTER II

Characteristics of the tomato chromoplast revealed by proteomic analysis

Cristina BARSAN[1,2,a], Paloma SANCHEZ-BEL[1,2,a], Cesar ROMBALDI[3,a], Isabel EGEA[1,2] Michel ROSSIGNOL[4,5], Marcel KUNTZ[6], Mohamed ZOUINE[1,2], Alain LATCHE[1,2], Mondher BOUZAYEN[1,2] and Jean-Claude PECH[1,2* a] Participated equally to the work

Journal of Experimental Botany, 2010, vol. 61: 2413-2431

ABSTRACT

Chromoplasts are non-photosynthetic specialised plastids that are important in ripening tomato fruit (*Solanum lycopersicum*) since, among other functions, they are the site of accumulation of coloured compounds. Analysis of the proteome of red fruit chromoplasts revealed the presence of 988 proteins corresponding to 802 Arabidopsis unigenes, among which 209 had not been listed so far in plastidial data banks. These data revealed several features of the chromoplast. Proteins of lipid metabolism and trafficking were well represented, including all the proteins of the lipoxygenase pathway required for the synthesis of lipid-derived aroma volatiles. Proteins involved in starch synthesis co-existed with several starch-degrading proteins and starch excess proteins. Chromoplasts lacked proteins of the chlorophyll biosynthesis branch and contained proteins involved in chlorophyll degradation. None of the proteins involved in the thylakoid transport machinery were discovered. Surprisingly, chromoplasts contain the entire set of Calvin cycle proteins including Rubisco, as well as the oxidative pentose phosphate pathway (OxPPP). The present proteomic analysis, combined with available physiological data, provides new insights into the metabolic characteristics of the tomato chromoplast and enriches our knowledge of non-photosynthetic plastids.

Introduction

Fruit ripening involves a series of biochemical and physiological events resulting in organoleptic changes in texture, aroma and colour. In many fruit one of the most important and more visible changes corresponds to the loss of chlorophyll and the synthesis of coloured compounds such as carotenoids. Carotenoids accumulate in chromoplasts that are non-photosynthetic plastids often

present in flowers and fruit and also occasionally found in roots and leaves. In both flowers and fruit, they serve the reproduction strategy of the plant by attracting pollinators and animals that disperse the seeds. In tomato, which is widely used as a model fruit, it is not clear whether chromoplast differentiation is a consequence of the ripening process or whether chromoplasts play a role in the onset of the ripening process. It is well known that, in climacteric fruit, the ripening process is triggered by the plant hormone ethylene (Lelièvre *et al.,* 1997; Giovannoni, 2001) and fruit physiologists have contributed to the elucidation of the mechanisms governing the mode of action of ethylene and the accumulation of metabolites responsible for important quality attributes (e.g. aromas, vitamins and antioxidants). In recent years, a number of genes and proteins involved in the fruit ripening process have been isolated through the implementation of modern genomics (Moore *et al.,* 2002) and proteomics (Faurobert *et al.,* 2007). However, little attention has been paid to the mechanisms of fruit ripening at the subcellular level. For instance, the detailed functioning of chromoplasts are not well understood despite their crucial role in the generation of major metabolites that are essential for the sensory and nutritional quality of fruit. A combination of experimental and bioinformatics data have estimated the size of the plastid proteome to be around 2700 proteins, amongst which more than 95% are imported (Soll, 2002; Millar *et al.,* 2006). The sequencing of the tomato chloroplast genome established that it contains 114 genes (Kahlau *et al.,* 2006) and that the differentiation of the chromoplast does not involve re-arrangements of the plastid genome (Hunt *et al.,* 1986). Therefore, knowledge of the plastidial genome provides little informatiion about the proteins that reside in the chromoplast and that underlie the wide variety of metabolic and regulatory events associated with this organelle. Major programmes devoted to the generation of ESTs and to the sequencing of the genome have been initiated with tomato as a model plant, but the accumulation of data on global gene expression and on genome sequences remains of limited value in understanding the function of chromoplasts. In addition, these sequencing programmes can address neither the post-translational protein modifications nor the subcellular localisation of the biosynthetic pathways. For these reasons, high-throughput proteomics associated with bioinformatics represents the most appropriate strategy towards identifying the protein components of the chromoplast and hence uncovering the multiple functions of the organelle. Comprehensive proteome information is expected to bring new insights into processes such as intracellular protein sorting as well as biochemical and signalling pathways. To date, the most important progress in relation to the

plastid proteome has been made for chloroplasts (Kleffmann *et al.,* 2004; Zybailov *et al.,* 2008) and this analysis includes sub-organelle protein localization for the thylakoid and lumen, (Peltier *et al.,* 2002; Schubert *et al.,* 2002), the stroma (Peltier *et al.,* 2006), the envelope (Ferro *et al.,* 2003) and plastoglobules (Ytterberg *et al.,* 2006). Advances have also been made in protein targeting mechanisms (Zybailov *et al.,* 2008; Jarvis, 2008). The proteomes of heterotrophic plastid types have been studied less extensively and are restricted to rice etioplasts (von Zychlinski *et al.,* 2005), wheat amylopasts (Andon *et al.,* 2002; Balmer *et al.,* 2006) and tobacco proplastids (Baginsky *et al.,* 2004). An analysis of the bell pepper chromoplast identified 151 proteins using MS/MS tandem mass spectrometry (Siddique *et al.,* 2006). Protein profiling of plastoglobules from pepper fruit chromoplasts and the Arabidopsis leaf chloroplast has also been performed, yielding around 20 proteins (Ytterberg *et al.,* 2006). In the present work, we have isolated chromoplasts from ripe tomato fruit and sequenced the soluble and insoluble protein fractions using LC-MS/MS LTQ-Orbitrap technology. This proteomic study substantially enlarges the number of chromoplastic proteins identified so far and provides new information on metabolic and regulatory networks in heterotrophic chromoplasts.

1. Material and methods

1.1. Isolation of tomato chromoplasts

Approximately 300 g of tomato fruits (*Solanum lycopersicum* cv MicroTom) were picked 10 days after breaker. The seeds and the gel were eliminated and the pericarp was cut in small pieces. The pieces of pericarp were rinsed twice in ice-cold extraction buffer (HEPES 250 mM, sorbitol 330 mM, EDTA 0.5 M, β-mercaptoethanol 5m M pH 7.6). The whole suspension was then put in a cold Waring Blendor and blended by a short pulse at minimum speed. After filtering through two layers of gauze and 60 μm nylon net, the filtrate was centrifuged at 4°C, 4000 rpm for 5 min, the supernatant discarded and the pellet recovered in 50μL of extraction buffer. The pellet was the loaded onto a gradient made of three layers of 0.5 M, 0.9 M and 1.45 M sucrose and then centrifuged 45 min at 4°C at 62,000*g*. Western blot and microscopic observations indicated that intact chromoplasts were located at the interface between the 0.9 and 1.45 M sucrose layers.

1.2. Analysis of chlorophyll, carotenoids and tocopherols

The content in carotenoids, chlorophyll and tocopherols of tomatoes at breaker + 10 days evaluated as described by Fraser *et al.,* (2000).

1.3. Western blot analysis

In order to assess the degree of enrichment of the chromoplast fraction, western blot analysis was performed using polyclonal antibodies at appropriate dilution against chloroplastic photosystem II D1 protein (psbA/D1, 32 kD, at 1:10 000 dilution) and Rubisco large subunit (RubcL, 5 kD, 1:50 000), cytosolic sucrose phosphate synthase (SPS, 120 kD, 1:1 000), mitochondrial voltage-dependent amino-selective channel protein 1 (Vdac1, 29 kD, 1:1 000), and vacuolar ATPase (V-ATPase, 26-27 kD, 1:5 000) from Agrisera® and cell-wall proteins, polygalacturonase (PG, 41-43 kD, 1:5 000) and pectin methyl esterase (PME, 31 kD, 1:5 000) generated by us from recombinant proteins corresponding to X77231 and X95991 cDNA respectively. Total fruit proteins were extracted from fruit harvested at 10 days after breaker and ground in liquid nitrogen according to Campbell *et al.,* (2003). Fruit and chloroplast proteins were separated by SDS-PAGE, transferred to a nitrocellulose membrane (Hybond ECL, GE Healthcare®)(FigX), treated with blocking TTBS buffer (20 mM Tris, 137 mM NaCl, 0.1% v/v Tween-20, pH 7.6, containing 2% w/v of ECL Advancing Blocking®), and subsequently incubated for 1 h with polyclonal antibodies diluted as indicated above in TTBS. Detection was performed with a peroxidase labelled anti-rabbit antibody (GE Healthcare®), diluted 1:50,000 in TTBS, and the membranes were developed using the GE Healthcare® Kit (ECL Advancing Western® blotting detection reagents). Western blot were made in duplicate from two chromoplasts isolations

1.4. Fractionation of proteins

In order to increase the access to low-abundant proteins and therefore improve the efficiency of the proteomic analysis, chromoplasts were sub fractionated into so-called soluble and insoluble factions. Chromoplasts of the 0.9 M-1.45 M sucrose interface were broken by osmotic shock adding 1:1(v/v) 1M HEPES buffer complemented with 2mM DTT, followed by freeze/thawing and homogenization in a Potter-Elvehjem tissue grinder. The soluble fraction was obtained by two consecutive ultra-centrifugations of the chromoplast extract at 100,000*g* for 1h at 4°C. The two supernatants were mixed and precipitated overnight in methanol 1:6 (v/v) at -20°C, then

centrifuged at 16,000*g*, for 30 min at 4°C and the precipitate incubated for two hours at room temperature in 4x Laemmli buffer [250 mM Tris-HCl, pH 6.8, 40% glycerol (v/v), 8% SDS (w:v), 0.01% bromophenol blue(w/v)]. The pellet corresponding to the insoluble fraction was incubated in 4x Laemmli buffer overnight at room temperature. Proteins were quantified according to Bradford after TCA precipitation and re-solubilization in 0.1N NaOH.

1.5. ***SDS-PAGE***

Samples (around 50 μg proteins) of soluble and insoluble fractions were boiled in SDS-sample buffer and then subjected to SDS-PAGE in 12% (w/v) polyacrylamide gel. After electrophoresis proteins were stained with PageBlue™ Protein Staining Solution (Fermentas).

1.6. LC-MS/MS as analytical method for the identification of chromoplast proteins

Each lane (soluble and insoluble fractions) from 1-D gel electrophoresis separation was cut into 15 homogenous slices that were washed twice in 100 mM ammonium bicarbonate/acetonitrile (1:1), 15 min at 37°C. Proteins were digested by incubating each gel slice with 0,5 μg of modified sequencing grade trypsin (Promega, Madison, WI, USA) in 50 mM ammonium bicarbonate, overnight at 37°C. The resulting peptides were extracted following established protocols (Wilm *et al.*, 1996) and final solution was dried in speed-vac.

The trypsin digests were reconstituted in 18μL of 5% acetonitrile, 0.05% trifluoroacetic acid then 5 μL were analysed by nanoLC-MS/MS using an Ultimate 3000 system (Dionex, Amsterdam, the Netherlands) coupled to an LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The peptide mixture was loaded on a C18 precolumn (300 μm ID x 15 cm PepMap C18, Dionex) equilibrated in 95% solvent A (5% acetonitrile, 0.2% formic acid) and 5% solvent B (80% acetonitrile, 0.2% formic acid). Peptides were eluted using a 5 to 50% gradient of solvent B during 80 min at 300 nL/min flow rate. Data were acquired with Xcalibur (LTQ Orbitrap Software version 2.2, Thermo Fisher Scientific). The mass spectrometer was operated in the data-dependent mode and was externally calibrated. Survey MS scans were acquired in the orbitrap on the 300-2000 m/z range with the resolution set to a value of 60,000 at m/z 400. Up to 5 most intense multiply charged ions (2+, 3+ and 4+) per scan were CID fragmented in the linear ion trap. A dynamic exclusion window was applied within 60 sec. All tandem mass spectra were collected using normalized collision energy of 35%, an isolation

window of 4 m/z, and 1 μscan. Other instrumental parameters included maximum injection times and automatic gain control targets of 250 ms and 500,000 ions for the FTMS, and 100 ms and10, 000 ions for LTQ MS/MS, respectively.

1.7. Database search and data analysis

Data were analyzed using Xcalibur software (version 2.0.6, Thermo Fisher Scientific) and MS/MS centroid peak lists were generated using the extract_msn.exe executable (Thermo Fisher Scientific) integrated into the Mascot Daemon software (Mascot version 2.2.03, Matrix Sciences). Dynamic exclusion was employed within 60 seconds to prevent repetitive selection of the same peptide. The following parameters were set to create peak lists: parent ions in the mass range 400-4,500, no grouping of MS/MS scans, and threshold at 1,000. A peaklist was created for each fraction (*i.e.* each gel slice) analyzed and individual Mascot searches were performed for each fraction. Data were searched against the EST-tomato SGN database (2006-07-05) containing 208974 sequences and 59634226 residues (http://www.sgn.cornell.edu/). Mass tolerances in MS and MS/MS were set to 5 ppm and 0.8 Da, respectively, and the instrument setting was specified as "ESI Trap". Trypsin was designated as the protease (specificity set for cleavage after K or R), and one missing cleavage was allowed. Oxidation of methionine, deamidation of asparagine and glutamine were searched as variable modifications, no fixed modification was set. Mascot results were parsed with the home made and developed software MFPaQ version 4.0 (Mascot File Parsing and Quantification) (Bouyssié *et al.*, 2007). Protein hits were automatically validated if they were identified with at least either: (i) one top ranking peptide with a Mascot score of more than 36.7 (for 36 proteins corresponding to this situation the spectrum of fragmentation is given in Annex; (ii) two top ranking peptides each with a Mascot score of more than 26.3; or (iii) three top ranking peptides each with a Mascot score of more than 22.9. To evaluate false positive rates, all the initial database searches were performed using the"decoy" option of Mascot, *i.e.* the data were searched against a combined database containing the real specified protein sequences (target database, EST-tomato SGN database) and the corresponding reversed protein sequences (decoy database). MFPaQ used the same criteria to validate decoy and target hits, calculated the False Discovery Rate (FDR = number of validated decoy hits / (number of validated target hits + number of validated decoy hits) x 100) for each gel slice analyzed, and made the average of FDR for all slices belonging to the same gel lane (*i.e.*to

the same sample). FDRs were below 1.6%. From all the validated result files corresponding to the fractions of a 1D gel lane, MFPaQ was used to generate, a unique non-redundant list of proteins that were identified and characterized by homology-based comparisons with the Arabidopsis database (TAIR8).

1.8. Database comparative proteomics, targeting predictions and functional classification

Proteins description were performed using annotations associated with each protein entry and through homology-based comparisons with the TAIR8 protein database (http://www.arabidopsis.org/) using BasicLocal Alignment Search Tool BLASTX (Altschul et al., 1990) with an e-value cut-of of 1e-5 to avoid false positives, and linked. MapMan Bins were used for functional assignments (http://mapman.mpimp-golm.mpg.de/). The protein list was compared to three plastidial or subcellular localization databases: Plprot (Kleffmann *et al.*, 2006), PPDB (Sun *et al.*, 2008) and SUBA (Heazlewood *et al.*, 2007). Predictions of subcellular localization were undertaken using TargetP (Emanuelsson *et al.*, 2000; http://www.cbs.dtu.dk/services/TargetP/), Predotar version 0.5 (http://www.inra.fr/predotar/) and iPSORT (http://hc.ims.u-tokyo.ac.jp/iPSORT/). Predictions were made on the basis of tomato proteins when harbouring an N-terminal sequence. Otherwise predictions were made using Arabidopsis homologs. Homology search with plastidial proteins from AT, tobacco, rice, wheat and chromoplastic proteins from pepper was determined by homology-based comparisons with the TAIR8 protein database (http://www.arabidopsis.org/). Sequence data arise from Von Zyklinski *et al.*, (2005) for rice etioplast, Baginski *et al.*, (2004) for tobacco proplastids, Siddique *et al.*, (2006) for pepper chromoplasts, Zybailov *et al.*, (2008) for Arabidopsis chloroplasts and Balmer *et al.*, (2006) for wheat amyloplasts.

2. Results and discussion

2.1. Isolation of chromoplasts from red tomato fruit

The pellet of chromoplasts recovered as described in “Material and Methods” was loaded onto a discontinuous gradient comprising 0.5, 0.9 and 1.45 M sucrose (**Fig 1A**). Plastidial photosystem II D1 protein (psbA/D1) and mitochondrial voltage-dependent amino-selective channel protein 1 (Vdac1) marker proteins were detected by western blotting (**Fig 1B**). The presence of several bands cross-reacting with the anti-psbA/D1 antibodies may correspond to various forms of the

protein during its processing and/or possible psbA/D1 complexes with other membrane proteins. The psbA/D1 protein is membrane-embedded in the large photosystem II complex (Campbell *et al.*, 2003). In the present proteomic analysis, it was only found in the insoluble fraction (Supplemental Table S2). The layer of plastids at the 0.9/1.45M interface was devoid of mitochondrial contamination and was assessed for purity using polyclonal antibodies against marker proteins of different cell compartments. As expected, proteins isolated from chromoplasts reacted with the anti RbcL antibodies (**Fig. 1C**). Proteins predicted to be located in the vacuole, cell wall, cytosol and mitochondria could not be detected. Interestingly, the antibodies were able to detect all these marker proteins in total protein extracts of breaker + 10 tomato fruit (**Fig. 1C**). These data indicate that the chromoplast preparation used for the subsequent proteomics analysis was of high purity.

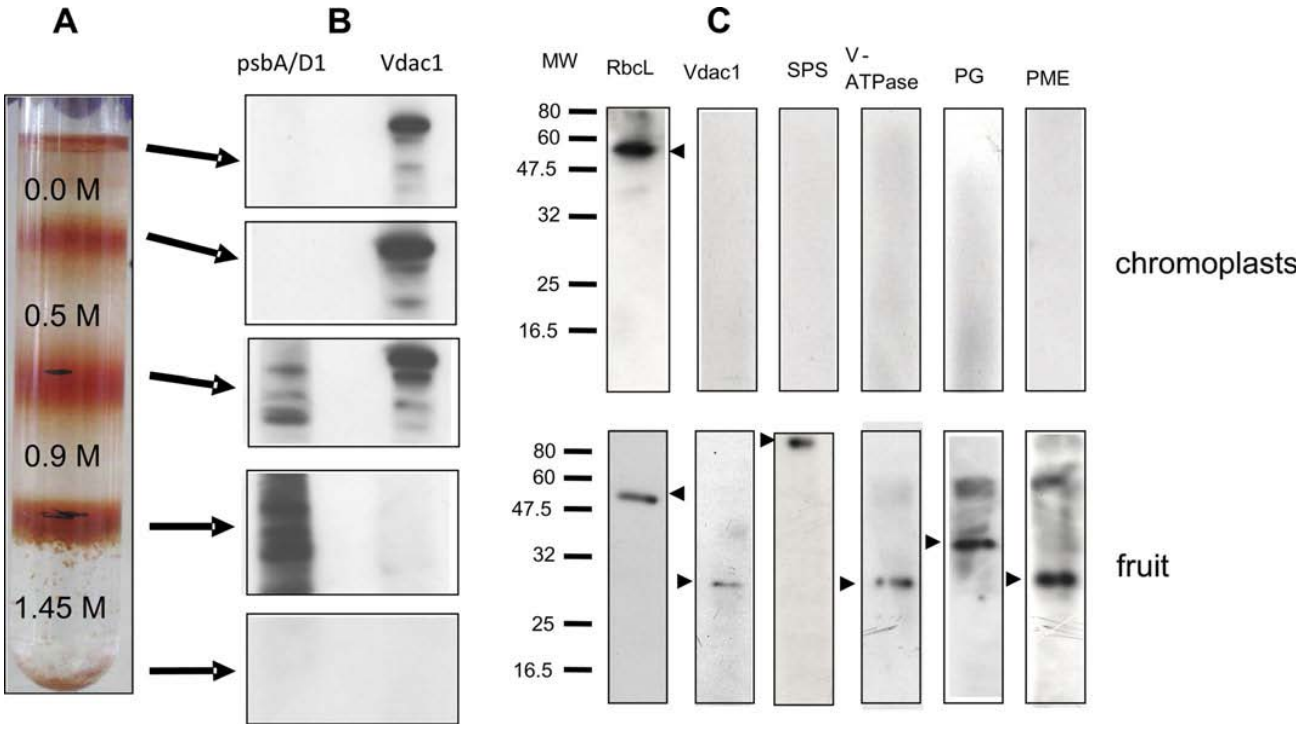


Figure 1: Isolation, purity control and fractionation of tomato fruit chromoplasts. A: Separation of chromoplasts on a discontinuous sucrose gradient (0.5, 0.9 and 1.45 M); B: Western blots for assessment of the purity of fractions at different interfaces of the sucrose gradient using antibodies for the plastidial PsBa/D1 and mitochondrial Vdac1 maker proteins; C: Western blots for assessment of the purity of chromoplasts as compared to whole fruit proteins using antibodies against plastidial large Rubisco subunit (Rbcl), mitochondrial voltage-dependent amino-selective channel protein 1 (Vdac1), cytosolic sucrose phosphate synthase (SPS), vacuolar ATPase (V-ATPase) and cell wall polygalacturonase (PG) and pectin methyl esterase (PME). Arrows indicate the actual molecular weight of the marker proteins.

2.2. ***Curation of isolated proteins by comparing with plastid data banks and by using predictors of subcellular localization***

Western blot data indicated that there was little contamination. However proteomic analysis revealed proteins that had not yet been annotated as plastidial and were absent from plastid data banks. We therefore curated the list of proteins by comparing with three plastid data banks (SUBA, PPDB and PlProt) and by using three targeting preditors (Target P, Ipsort, and Predotar). The final list, comprising 988 tomato unigenes corresponding to 802 Arabidopsis unigenes is given in **Annex.** Amongst the 988 proteins, 360 were found in the so-called "soluble" fraction extracted with the HEPES-DTT buffer, 170 in the so-called "insoluble" fraction solubilised with the Laemmli-SDS buffer and 458 in both fractions.

Figure 2 shows that 765, 506 and 332 chromoplast proteins corresponding to tomato unigenes were annotated in the SUBA, PlProt and PPDB libraries, respectively. However, 209 proteins revealed by proteomic analysis were not in the data banks, but were predicted as being plastidial by at least one of the three targeting predictors. They can therefore be considered as novel plastidial proteins and have been overlined in **Annex.**

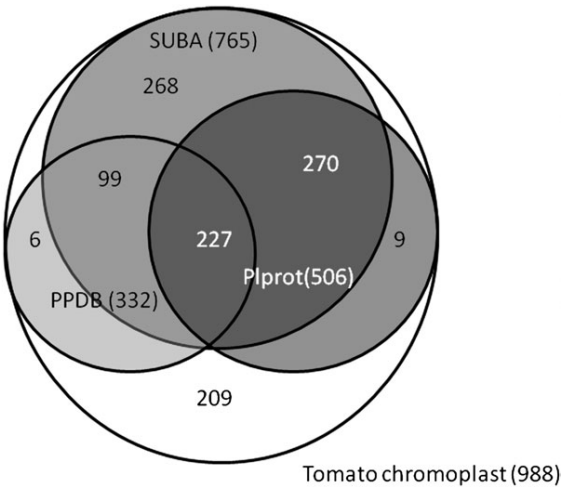


Figure 2: Venn diagram showing the presence of tomato chromoplastic proteins in plastidial databases: SUBA, PPDB and Plprot. Note that 209 proteins of the tomato chromoplast proteome were not listed in the databases. Comparison has been made on the basis of Arabidopsis annotations taking into account all tomato unigenes.

When comparing the tomato chromoplast proteome identified here with the proteome of other plastids on the basis of unique AT proteins (**Fig. 3**) it appears that the number of plastidial proteins identified in the present study (988) is of the same order of magnitude as the Arabidopsis

chloroplast proteome (1280), but higher than the proteome of wheat amyloplasts (289), rice etioplasts (240) tobacco proplastids (168) and pepper chromoplasts (151). The size of the AT plastid proteome has been estimated as approximately 2700 proteins (Millar *et al.,* 2006), indicating that we are still far from covering all chromoplastic proteins. Despite the heterogeneity in the total number of proteins identified in each proteome, it appears (**Fig. 3**) that 192 (66%) proteins of the wheat amyloplast, 577 (45 %) of the Arabidopsis chloroplast, 160 (66%) of the rice etioplast, 110 (65 %) of the tobacco proplastid and 108 (71%) of the pepper chromoplast were also present in the tomato chromoplast.

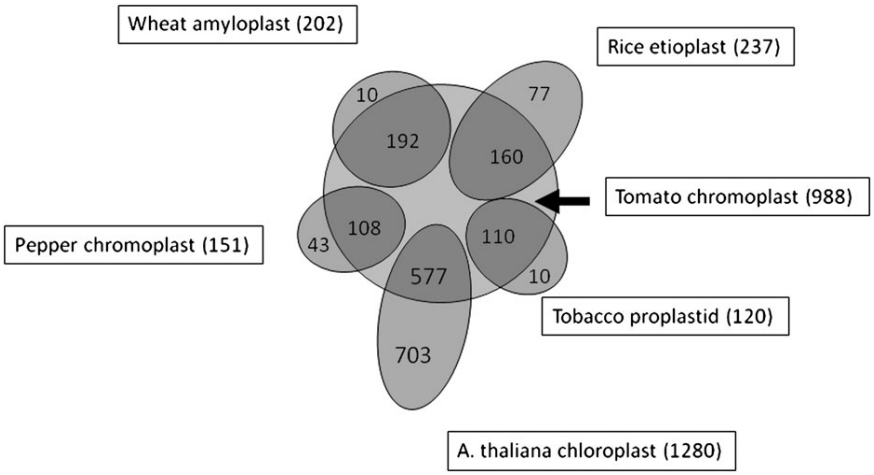


Figure 3: Diagram showing a comparison of tomato chromoplastic proteome with other plastidial proteomes. Data arise from von Zyklinski *et al.*, (2005) for rice etioplast, Baginski *et al.*, (2004) for tobacco proplastids, Siddique *et al.*, (2006) for pepper chromoplasts, Zybailov *et al.*, (2008) for Arabidopsis chloroplasts and Balmer *et al.*, (2006) for wheat amyloplasts. Comparison has been made on the basis of unique Arabidopsis annotations taking into account all unigenes.

Classification of the identified proteins according to MapMan allows an overview of the abundance of proteins in the various functional classes (Fig. 4). Apart from non-assigned proteins, the functions corresponding to the highest number of proteins are, by decreasing order of importance, protein-related processes, photosynthesis, amino acid metabolism and lipid metabolism.

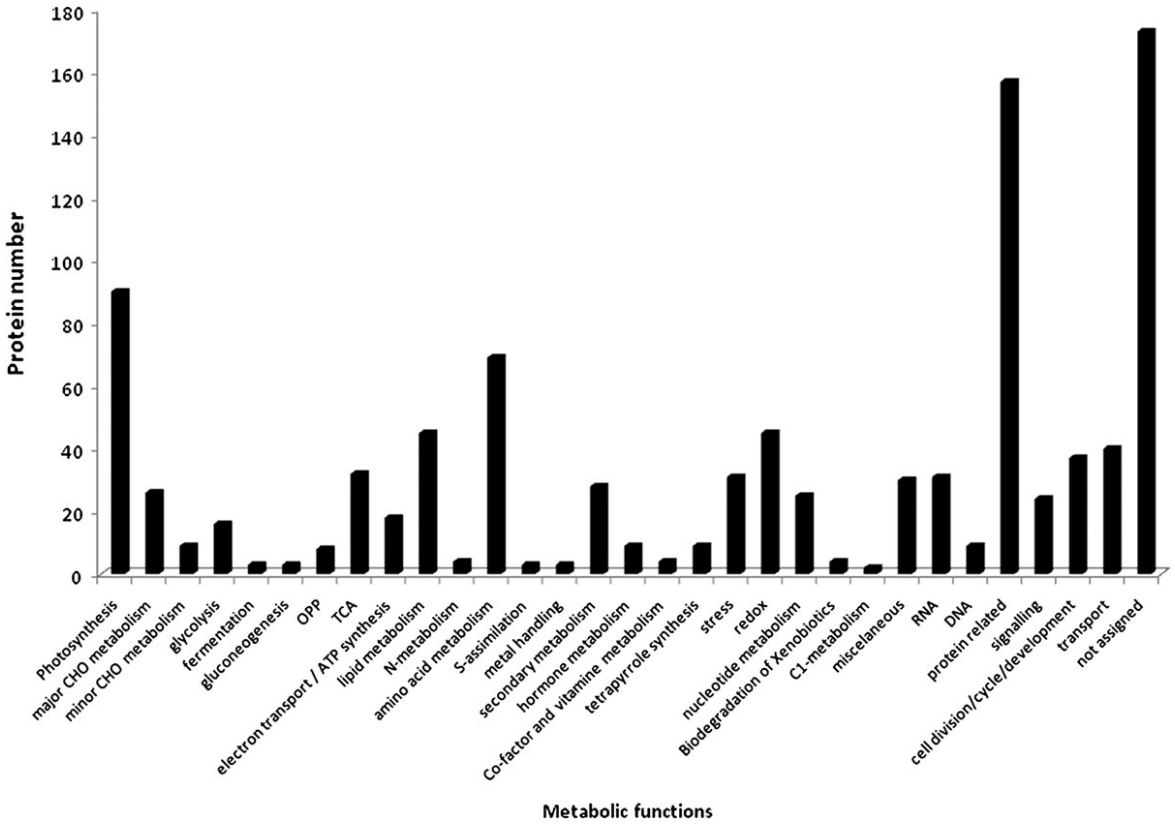


Figure 4: Functional classification of tomato chromoplast proteins. Proteins were assorted to their putative function by using the MapMan software (Thimm *et al.*, 2004 and http://mapman.mpimp-golm.mpg.de/).

2.3. Proteins encoded by the plastid genome

The tomato chloroplastic genome comprises 84 conserved open reading frames (Kahlau *et al.*, 2006). Amongst the 84 proteins, 22 have been found in our chromoplastic proteome (**Table 1**), including 3 proteins of Photosystem II, 1 of photosystem I, 2 cytochrome B6/f proteins, 4 ATP synthases, 1 protein of the Calvin cycle (Rubisco), 8 ribosomal proteins, 1 protein involved in protein degradation and 1 acetyl CoA carboxylase. We also identified the Ycf2 protein, which corresponds to the largest chloroplast genome-encoded protein. The *Ycf2* gene is highly expressed in chromoplasts during ripening (Richards *et al.*, 1991). Its function is not related to photosynthesis and is currently unknown. It has been shown that the Ycf2 protein plays a vital role in the plant cell (Drescher *et al.*, 2000). No RNA polymerase was detected, probably because of its low abundance.

Table 1: **List of proteins encoded by the tomato plastid genome encountered in the tomato chromoplast proteome**. Classification has been made according to MapMan.

PLASTID GENOME ENCODED PROTEINS	GI	PLASTID GENOME ENCODED PROTEINS	GI
PSII		**Protein.synthesis.ribosomal protein**	
chlorophyll binding protein psbA/D1	gi|89280615	ribosomal protein S11	gi|89280668
photosystem II 44 kD protein	gi|89280631	ribosomal protein S16	gi|89280617,gi|89280620
photosystem II 47 kD protein	gi|89280661	ribosomal protein S3	gi|89280673
PSI		ribosomal protein S4	gi|89280637
photosystem I P700 apoprotein A2	gi|89280634	ribosomal protein S8	gi|89280670
PS.lightreaction.cytochrome b6/f		ribosomal protein L16	gi|89280672
cytochrome f	gi|89280648	ribosomal protein L22	gi|89280674
cytochrome b6	gi|89280665	**Protein.degradation**	
PS.lightreaction.ATP synthase		ATP-dependent Clp protease	gi|89280660
ATP synt CF0 β sub	gi|89280621,gi|8928062	**Protein assembly and cofactor ligation**	
ATP synthase CF1 epsilon subunit	gi|89280641	Ycf2	gi|89280678
ATP synthase CF1 β chain	gi|89280642	**Lipid biosynthesis**	
PS.calvin cyle		acetyl-CoA carboxylase βsubunit ACCD	gi|89280644
Rubisco large subunit	gi|89280643		

2.4. Photosynthesis and Calvin cycle

A number of proteins involved in the PSI and PSII photosystems, in light reactions and in photorespiration were detected (**Table 2**), corresponding to 22% and 39% of the PSI and PSII proteins of the Arabidopsis chloroplast, respectively. Notably, the psbA/D1 protein, part of the core of photosystem II, has been shown to undergo rapid light-dependent degradation in chloroplasts (Mattoo *et al.*, 1984; Edelman and Mattoo, 2008). The small plastid-encoded, and the large nuclear-encoded Rubisco were also present. This is not surprising since they have also been found in non-photosynthetic wheat rice amyloplasts (Balmer *et al.*, 2006) and rice etioplast proteomes (von Zychlinski *et al.*, 2005). The persistence of photosynthetic proteins and active Rubisco has already been reported for late stages of tomato ripening (Bravdo *et al.*, 1977; Piechulla *et al.*, 1987). In addition, a 32 kD "Qb binding" protein, a plastocyanin (Piechulla *et al.*, 1987), the 68 kD subunit of PSI complex Cytf and a CF1ATPase subunit (Livne and Gepstein, 1988) have been detected by western blot analysis in ripe tomato fruit. The homologs of 9 of the photosynthetic apparatus proteins have been detected in the plastoglobules of Arabidopsis and one in the plastoglobules of pepper chromoplasts (Ytterberg *et al.*, 2006). Since plastoglobules cannot be considered as a site for photosynthesis, it is probable that either full size or segments of

non-functional proteins are stored in the plastoglobules after disintegration of photosynthesis complexes in the chromoplast. The absence of a large number of proteins involved in PSI and PSII could be related to an autophagy process similar to that described for senescent leaves. Interestingly, the SEN1 gene, described as being involved in autophagy (Wada *et al.*, 2009) has also been found here.

Chemical analysis performed on fruit at the breaker + 10 stage of ripening indicated undetectable levels of chlorophyll a and b. Consistent with the absence of chlorophyll in the chromoplasts, all steps of the chlorophyll biosynthesis branch were lacking (**Figure 5**), including the magnesium-chelatase, which is at the cross-road of the branch.

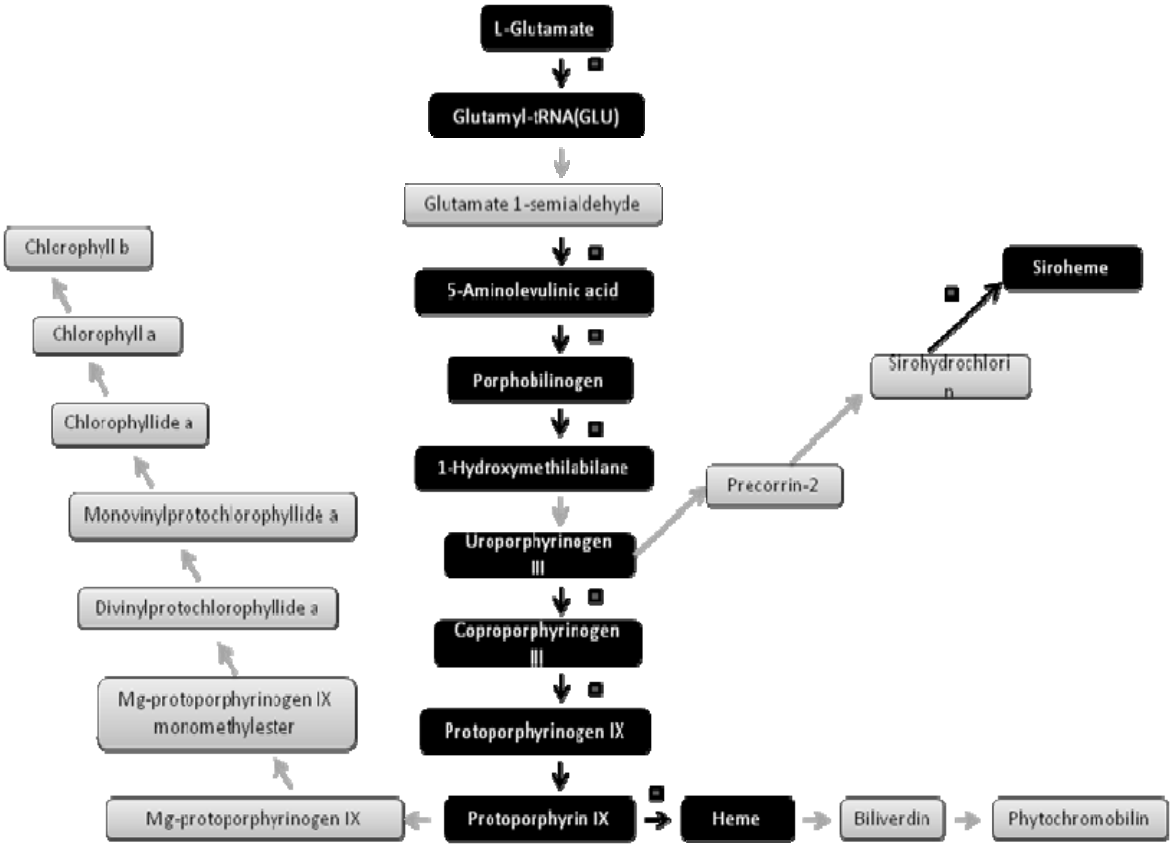


Figure 5: Status of the tetrapyrrole biosynthetic pathway in tomato chromoplasts. Most of the enzymes involved in the "trunk" pathway from L-glutamate to protoporphyrin IX were detected, while none of the enzymes of the "chlorophyll branch" were encountered. One protein was detected in each of the "siroheme and heme branches". Proteins present in the chromoplast proteome are mentioned by their SGN code.

This is consistent with the absence of photosynthetic activity in fruit at this stage of development (Piechulla *et al.,* 1987). Only proteins leading to the synthesis of protoporphyrin IX were found, with the exception of glutamyl tRNA reductase. Among the 3 proteins of the heme-derived pathway leading to phytochromobilin, involved in phytochrome synthesis and described as plastid-localized (Terry and Lagarias, 1991**),** only the protein of the first step was recovered in the chromoplast proteome. Concerning the protoporphyrin pathway, one protein out of 3 of the siroheme branch was detected. This branch provides the cofactor for sulfite reductase, involved in the assimilation of sulfur and its incorporation into sulfur amino acids, as well as for nitrite reductase, involved in the assimilation of nitrogen.

Interestingly, the chromoplast proteome comprises several proteins known to participate in chlorophyll catabolism. These include proteins directly involved in the breakdown of chlorophyll, pheophytinase (Schelbert *et al.,* 2009) and pheophorbide a oxygenase (Pruzinska *et al.,* 2005) and a regulator of pheophorbide a oxygenase, the stay-green protein, sgr1 (Ren *et al.,* 2007). The presence of these proteins in fully-developped chromoplasts, assuming they are enzymatically active, indicates that the chromoplast could comprise chlorophyll breakdown processes similar to those occuring during the senescence leaf chloroplasts (Thomas *et al.,* 2009).

Most of the proteins of the Calvin cycle were identified in the tomato chromoplast including Rubisco. Four different proteins of the small nuclear-encoded subunits of Rubisco, probably encoded by four different genes (proteins annotated as 3B subunits are in fact different) and 3 fragments of the plastid-encoded large subunit have been found. Also, a Rubisco activase, a Rubisco large subunit N-methyltransferase and two chaperonins (60α and 60β) were detected. This means that all components necessary for Rubisco activity are present. Almost all proteins of the OxPPP pathway are represented in the chromoplast proteome (**Table 2**), consistent with the presence of active OxPPP in ripening fruit and in pepper fruit chromoplasts (Thom et al., 1998). Although proteome analysis alone cannot provide evidence of the functionality of the Calvin Cycle, the persistence of all the proteins of this pathway suggests a possible role in metabolic adjustments that would provide not only reductants but also precursors of nucleotides (from ribose-5-phosphate) and aromatic amino acids (from erythrose-4-phosphate) to allow the OxPPP cycle to function optimally. Alternatively, the presence of the Calvin cycle part of the photosynthesis machinery may simply represent a left-over corresponding to the recovery of the

photosynthetic activity required for converting the chromoplast back to the chloroplast. This has already been observed in many plant tissues, including fruit (Hudák *et al.*, 2005).

2.5. Carbohydrate metabolism

Sugars derived from photosynthesis within the fruit are extremely limited at the approach of fruit maturity. The bulk of sugar accumulation comes mainly from transport through the phloem. The chromoplast has the potential to translocate sugars via a membrane located glucose 6-phosphate transporter and triose phosphate/phosphoenol pyruvate translocator. Due to the absence of photosynthetic activity the reducing power of the chromoplast may be satisfied by the light-independent production of NADPH through the glucose-6-phosphate dehydrogenase (G6PDH) and 6-phospho gluconate dehydrogenase (6PGDH) proteins of the oxidative pentose phosphate pathway, OxPPP (Kruger and van Schaewen, 2003). Previous biochemical data have characterized functional OxPPP and import of G6P in isolated sweet pepper (Thom et al., 1998) and buttercup (Tetlow *et al.,* 2003) chromoplasts. In addition to proteins of the OxPPP, it was mentioned earlier that the Rubisco protein and all proteins of the Calvin cycle have been found in tomato chromoplasts. If all these pathways are active, this could suggest that CO_2 generated by the OxPPP could be re-incorporated metabolically. Although such a possibility remains to be demonstrated in the chromoplast by enzymatic and metabolic analysis, re-assimilation of CO_2 generated from OxPPP by Rubisco has been clearly shown in non photosynthetic oil-accumulating seeds in order to sustain fatty acid biosynthesis (Schwender *et al.*, 2004).

Proteins of the starch biosynthesis pathway were also identified including soluble starch synthase, ADP-glucose pyrophosphorylase and 1,4-alpha-glucan branching protein. Starch grains have been observed in flower chromoplasts, especially in tissues grown in vitro (Keresztes and Schroth, 1979) indicating the functionality of the biosynthetic system. However, several proteins involved in starch degradation were also found such as α-amylase 3, β-amylase 3, glucan phosphorylase, phosphoglucan, water dikinase, disproportionating enzyme 1 and 2 and isoamylase 3, thus suggesting a rapid turnover of starch. In addition, starch excess proteins 1 and 4 (sex1 and 4) regulating starch accumulation were also present. Arabidopsis mutants for sex proteins accumulate an excess of starch (Yu *et al.,* 2001). Interestingly, neither starch-degrading protein nor starch excess protein have been reported in the proteome of wheat amyloplasts where a high accumulation of starch occurs (Balmer *et al.,* 2006).

2.6. Lipid synthesis and metabolism

Chromoplasts possess the entire metabolic equipment for the synthesis of 3-oxoacyl-ACP, the precursor of fatty acids (**Table 2** and **Figure 6**).

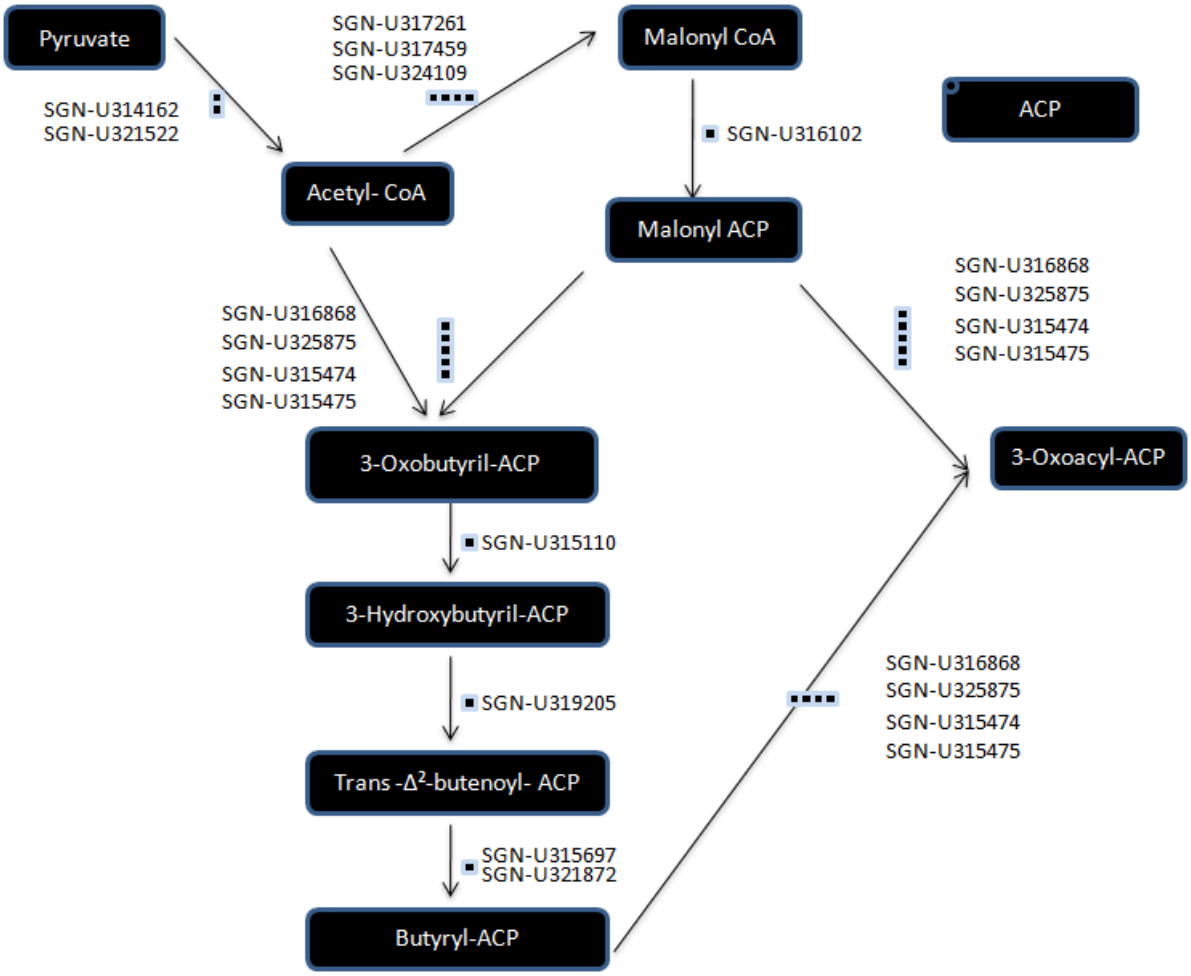


Figure 6 : Lipid biosynthesis pathway showing the presence of enzymes encountered in the tomato chromoplastic proteome. Note that all enzymes of the pathway are represented. Proteins are represented by their unigene SGN code.

Interestingly, almost all the subunits of acetyl CoA carboxylase were detected (three different proteins of the nuclear-encoded subunits corresponding to four different genes, CAC1, CAC2 and CAC3 and one plastid-encoded subunit, ACCD). Key proteins for the synthesis of phospholipids, glycolipids, sulfolipids and sterols were also identified (**Table 2**). If all these proteins are enzymatically active, these results indicate that the chromoplast has the ability to synthesize fatty acids and polar lipids such as sulfolipid and phosphatidylglycerol, probably in coordination with

the endoplasmic reticulum (Andersson *et al.,* 2007). The presence of the trigalactosyldiacylglycerol 2 protein, a permease-like component of an ABC-transporter involved in ER-to-thylakoid trafficking (Awai *et al.,* 2006) reinforces this hypothesis. A protein involved in vesicular transport from the inner envelope to thylakoids, "plastid transcriptionally active 4" (VIPP1) was also present (Kroll *et al.,* 2001), which is consistent with the presence of intense vesicular activity during chromoplast formation as shown by electron microscopy (Westphal *et al.,* 2001).

Many proteins involved in lipid metabolism were recovered in the chromoplast proteome (corresponding to category 11.9 in **annex**). Of special interest is the presence of all proteins potentially involved in the LOX pathway, leading to the generation of aroma volatiles, including phospholipase D α 1, lipoxygenase C (Chen *et al.,* 2004,) and hydroperoxide lyase. In addition, an alcohol dehydrogenase 2 capable of interconverting aldehydes and alcohols is present. This protein has been shown to participate in aroma formation in tomato (Speirs *et al.,* 1998). It is therefore possible to assign a role of the chromoplast in the synthesis of LOX-derived volatiles which are known to be synthesized at a high level in ripe red fruit (Birtic *et al.,* 2009) at a stage where LOX-C gene expression is still high (Griffiths et al., 1999).

2.7. *Proteins related to transcription, translation and posttranscriptional modifications*

This category comprises 121 proteins that have not been reported in Table 1 but are listed in Annex under the categories 27.1 to 29.2.5 according to MapMan. These proteins, potentially involved in transcription, translation, folding, assembly, turnover and protein storage, represent the major functional group found in chromoplasts (**Fig. 4**).

No RNA polymerase has been detected among the sequenced proteins encoded either by the nucleus or by the chromoplastic genome. This indicates that the transcriptional activity at this stage of ripening was probably very low. This is consistent with the progressive decline in the overall rate of RNA synthesis observed throughout chromoplast development in ripening tomato fruits (Marano and Carillo, 1992). The absence of detectable RNAse exonuclease II which is thought to participate in the RNA degradation pathway could account for higher stability of the RNA. Sustained transcriptional activity has been measured in chloroplasts (Briat *et al.,* 1982), but a 5 to 10-fold decrease in activity for most plastid genes was observed in chromoplasts (Deng and Gruissem, 1987). Other experiments did not report such major variations in the relative

transcription rate (Marano and Carrillo, 1992; Kahlau and Bock, 2008), except for the up-regulation of the *trnA* gene (encoding the tRNA-Ala) and the *rpoC2* gene (encoding an RNA polymerase subunit) and a significant up-regulation of the *acetyl-CoA carboxylase* gene (*ACCD*), the only plastid-encoded gene involved in fatty acid biosynthesis (Kahlau and Bock, 2008). The plastid-encoded ACCD was found in the tomato chromoplast proteome analyzed in this study. Seventeen transcription factors were detected (category 27.3 in **Annex**), nine of these having a plastid signal. This low number of these factors is probably related to the low number of chromoplastic genes requiring regulation. However they may play an important role in signaling of the nucleus to the plastids (anterograde signaling). Plastids have 70S ribosomes comprising 50S and 30S subunits (Yamaguchi and Subramanian, 2003). These ribosomal proteins are represented by 7 nuclear-encoded proteins of the 50S fraction and 9 of the 30S fraction (29.2.1.1 category in **Annex**), indicating that the translational machinery is present. However our data cannot tell whether this machinery is functional. The presence of 13 tRNA synthases or ligases (29.1 category), 8 elongation factors (29.2.4 category) and numerous chaperonins are additional indications of translational activity. As already found for bell pepper (Siddique *et al.*, 2006), tomato chromoplasts contain a translation inhibitor protein of the L-PSP type (SGN-U317502) presumed to have endonuclease activity towards mRNAs that might prevent the translation of certain proteins that are no longer required in chromoplast function. Most plastid genes are transcriptionally down-regulated during chromoplast development, especially photosynthesis-related genes. The *ACCD* gene, which is involved in fatty acid biosynthesis, is the only plastid-encoded gene showing stable expression in chromoplasts (Kahlau and Bock, 2008). Interestingly, functional ribosomes and translation activity have been observed in tobacco plants in which the plastid RNA polymerase genes have been disrupted (De Santis-Maciossek *et al.*, 1999). Therefore, the undetectable levels of RNA polymerase in tomato chromoplast seems compatible with the presence of active translational activity.

2.8. Amino acid metabolism

Four of the six proteins of the shikimate pathway (Herrmann and Weaver, 1999) have been identified: 3-deoxy-7-phosphoheptulonate synthase, shikimate 5-dehydrogenase, 3-phosphoshikimate 1-carboxyvinyltransferase and chorismate synthase. The final step of the pathway produces chorismate, the precursor of the aromatic amino acids phenylalanine, tyrosine

and tryptophan. The presence of this pathway within the chloroplast has already been suggested by Herrmann and Weaver (1999). Confirmation of the synthesis of the three amino acids in the chromoplast is provided by the fact that many of the proteins involved in the aromatic amino acid biosynthetic pathway are present in the tomato chromoplastic proteome, especially those of the final step: anthranilate synthase, anthranilate phosphorybosyltransferase, tryptophan indole-3-glycerol phosphate synthase, tryptophan synthase α and β subunit. The synthesis of methionine is known to be linked to the aspartate pathway and to the assimilation of sulfur and incorporation in cysteine (Hesse and Hoefgen, 2003). In the tomato chromoplastic proteome we have encountered almost all of the proteins of this pathway: homoserine kinase, threonine synthase, aspartate semialdehyde dehydrogenase and bi-functionnal aspartate kinase/homoserine dehydrogenase. The intracellular localisation of the final step of methionine synthesis is a matter of debate (Hesse and Hoefgen, 2003; Ravanel *et al.,* 2004). Among the chromoplastic proteins we have identified cystathionine beta-lyase, a clear indicator of the synthesis of methionine within the chromoplast. One Arabidopsis isoform was also found to be located in the chloroplast (Ravanel *et al.,* 2004). The presence of proteins involved in the early steps of sulfur assimilation and in the assimilation of ammonia in tomato chromoplasts is indicative of the capability to assimilate sulfur and ammonia.

2.9. Terpenoid metabolism

The large majority of the proteins of the Rohmer pathway (non-mevalonate or methylerythritol phosphate pathway) leading to the synthesis of terpenoid precursors in plastids are present in the tomato chromoplast (**Table 2**) except for the 4-diposphocytidyl-methylerythritol kinase. The mevalonate pathway is represented by one protein, acetoacetyl-CoA thiolase 2. Both the Rohmer and the mevalonate pathways lead to the formation of the C5 compounds isopentenyl diphosphate (IPP) and dimethyl allyl diphosphate which can be interconverted by IPP isomerase, also present in the tomato chromoplastic proteome. The following steps involve prenyl transferases. Two geranylgeranyl pyrophosphate (GGPP) synthase (synthesis of the C20 precusor of carotenoid, gibberellines and side chains of tocopherols, phytol and phylloquinone) and two geranylgeranyl reductases have also been identified. They could participate in the reduction of geranylgeranyl-chlorophyll to chlorophyll a and also of free geranylgeranyl diphosphate into phytyl diphosphate, which is used for chlorophyll, tocopherol and phylloquinone synthesis (Zybailov *et al.,* 2009).

Chemical analysis performed on fruit at breaker + 10 stage of ripening showed the prevalence of lycopene (all-trans and, as a minor form, a cis isomer). Beta-carotene, a compound tentatively identified as γ-carotene and lutein were also detected (in decreasing order of content), as well as traces of other compounds (data not shown). Almost all proteins dedicated to the biosynthesis of lycopene have been identified among the chromoplastic proteins: phytoene synthase 1, phytoene desaturase and two zeta-carotene desaturases. Interestingly tomato has two phytoene synthases, a chloroplastic PSY-2 which is expressed in green tissues and green fruit and a chromoplastic PSY-1 which strongly accumulates during fruit ripening (Fraser *et al.*, 1999). The presence of only PSY-1 in our set of proteins is therefore consistent with the metabolic data previously published. Surprisingly, no sequence for the plastid terminal oxidase (PTOX), a co-factor for carotene desaturases, has been identified, although data indicate that a mutant deficient in this protein is severely impaired in lycopene synthesis during tomato fruit ripening (Shahbazi *et al.*, 2007). The most likely explanation is that PTOX is present at such a low abundance that it has not been detected in our proteomic analysis. Downstream proteins, namely lycopene cyclases (lycopene β-cyclase, lycopene ε-cyclase and carotenoid hydroxylases (β-carotene hydroxylase and carotenoid ε-hydroxylase) were not identified and are therefore either totally absent or present at undetectable levels. This is in agreement with data showing that the accumulation of lycopene is due to a down-regulation of the genes encoding downstream proteins of this pathway (Ronen *et al.*, 1999) thus leading to a weak metabolic flux towards the synthesis of beta carotene and xanthophylls. However, we do find a zeaxanthine epoxidase, catalyzing the synthesis of violaxanthin, which is consistent with the presence of violaxanthin in tomato chromoplasts.

2.10. Biosynthesis of vitamins

It is known that the chloroplast is the site for the synthesis of thiamine, vitamin B1 (Julliard and Douce, 1991). However, none of the proteins involved in thiamine biosynthesis were detected in the tomato chromoplast proteome. Several proteins of riboflavine (vitamin B2) biosynthesis (Roje, 2007) have been identified in the tomato chromoplastic proteome: GTP cyclohydrolase II, 6,7-dimethyl-8-ribityllumazine synthase and lumazine-binding family protein similar to riboflavin synthase. These data confirm the predictions made so far by both experimental and bio-informatic analysis (Roje, 2007). Only one protein of the folate (vitamin B9) biosynthesis pathway has been encountered. The active protein catalyses the conversion of chorismate to para-

aminobenzoate: 4-amino-4-desoxychorismate lyase and is part of the pathway already known to be located in the plastids (Bedhomme *et al.,* 2005).

The early steps of the biosynthesis of the side chain of tocopherols (vitamin E), which are in common with other metabolic pathways (terpenes, sterols, carotenoids…), are present in the chromoplasts and discussed in the lipid section. Regarding the other branches of the pathway, our data show the presence of the methyltransferase converting methyl phytylquinol to the gamma/alpha-tocopherol branch (at the expense of the delta/beta-tocopherol branch) and the final protein of this branch, gamma-tocopherol methyltransferase activity, involved in conversion of gamma tocopherol to α-tocopherol formation. The latter protein has already been described in the *Capsicum* chromoplast (d'Harlingue and Camara, 1985). The apparently greater abundance of these two proteins from the tocopherol pathway may explain the large prevalence of alpha-tocopherol and to a lesser extent gamma-tocopherol, and the virtual absence of delta and beta-tocopherol in tomato chromoplast extracts. Chemical analysis performed on fruit at breaker +10 stage of ripening revealed the presence 2.25 $\mu g.g^{-1}$ FW of α-tocopherol, 0.15 $\mu g.g^{-1}$ of γ-tocopherol. The tocopherol cyclase, VTE1, which has been identified in chloroplast plastoglobules of Arabidopsis (Vidi *et al.,* 2006) has not been encountered in the tomato chromoplast proteome.

2.11. . Redox proteins

Reactive oxygen species appear to regulate carotenoid synthesis in chromoplasts (Bouvier *et al.,* 1998) and redox systems are considered to have several functions in the plastids, including plastoglobule protection, pathogen defence, stress response, protection against reactive oxygen species, signalling and energy (Foyer and Noctor, 2003). As many as 21 proteins of the ascorbate-glutathione cycle have been detected (Table 2). They include key components of the cycle such as stromal and thylakoid-bound L-ascorbate peroxidase, glutathione peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase. In addition, three types of peroxiredoxins and four types of thioredoxins have been identified. The thioredoxin reductase encountered in the chromoplast may be indicative of the presence of the so-called NADPH-dependent thioredoxin system. The number of proteins involved in redox reactions is also significative, including several catalases, superoxide dismutases, peroxidases not belonging to the class of ascorbate peroxidases and NADH-ubiquinone oxidoreductases. The proton-pumping

NADH:ubiquinone oxidoreductase, also called complex I, is the first of the respiratory complexes providing the proton motive force essential for the synthesis of ATP. The presence of a chemiosmotic ATP synthesis has been demonstrated in the chloroplast (Morstadt *et al.,* 2002) which is linked to a redox pathway and potentially involved in carotene desaturation and membrane energization. Closely related forms of this complex exist in the mitochondria of eucaryotes and in the plasma membranes of purple bacteria (Friedrich *et al.,* 1995). Such an important list of redox proteins indicates that chromoplasts have integrated antioxidant defense/protection machinery, similarly to chloroplasts (Giacomelli *et al.,* 2007) with sometimes dual targeting to the mitochondria (Chew *et al.,* 2003). In support of the presence of a functional redox system in chromoplasts, it has been demonstrated that the activity of superoxide dismutase and proteins of the ascorbate-glutathione cycle was up-regulated during ripening of pepper fruit (Marti et al., 2009).

2.12. Hormones

Proteins involved in the synthesis of several hormones have been encountered. Homologs of the alpha and beta subunits of anthranylate synthase of Arabidopsis are present in the tomato chromoplasts. They are involved in the biosynthesis of tryptophan and have been described as key elements in the regulation of auxin production. The encoding genes are ethylene responsive, which makes a link between ethylene and auxin (Stepanova *et al.,* 2005). IAA synthesis has been proposed to occur via a cytosolic tryptophan-dependent (indole-3-acetaldoxime) and a plastidial tryptophane–independent (indole-3-glycerophosphate) pathway. In the tomato chromoplast we have only encountered an indole-3-glycerol phosphate synthase involved in the tryptophan-independent patway (Ouyang et al., 2000). Two proteins of the ABA pathway have been identified: zeaxanthine epoxidase and short-chain dehydrogenase indicating that chromoplasts could be active in producing ABA. However, the absence of the 9-cis-epoxycarotenoid dioxygenase may be indicative of the low activity of the ABA biosynthetic pathway coincident with the decrease in ABA content well before the climacteric peak in tomato (Martinez-Madrid *et al.,* 1996). The three proteins involved in the early steps of the biosynthesis of jasmonates which are present in the chloroplast (Delker *et al.,* 2007) have also been identified in the tomato chromoplast: lipoxygenases 2 and 3, allene oxide synthase and allene oxide cyclase. The final steps occur in the peroxisome (Delker *et al.,* 2007). Proteins involved in the formation of the

gibberellin skeleton (ent-copalyl diphosphate synthase and ent-kaurene synthase) are known to be present in the chloroplast (Railton *et al.,* 1984). They were not found among the tomato chromoplastic proteins, suggesting the absence or low level of gibberellin biosynthesis.

2.13. Signaling elements

Two hexokinase1 homologs that could potentially participate in glucose signalling are present in the tomato chromoplast. In Arabidopsis, hexokinase1 has been located to the mitochondria (Rolland and Sheen, 2005) but in spinach hexokinase activity has been found in plastids (Wiese *et al.,* 1999). Chloroplast-to-nucleus signalling (retrograde signalling) can be mediated by reactive oxygen species (ROS), Mg-protoporphyrin IX as well as by secondary messengers such as Ca2+ (Surpin *et al.,* 2002). Many proteins involved in ROS have been identified. Mg-Protoporphyrin IX plays an important role in retrograde signalling (Strand *et al.,* 2003) by inhibiting the expression of the nuclear genes involved in photosynthesis. However, no magnesium-chelatase has been detected here indicating that the synthesis of Mg-protoporphyrin IX is probably not very active in chromoplasts. This is consistent with the fact that down-regulation of genes involved in photosynthesis occurs at early stages of fruit ripening. Several elements of the calcium signalling pathway have been encountered including calmodulin, calnexin and a calcium-binding EF hand family protein (**Table 2**).

2.14. Structural and building blocks

Seven out of the 10 tomato fibrillin-type lipid-associated proteins expected from genomic data (Laizet *et al.,* 2004) have been identified. A number of these proteins have been found in the chloroplast thylakoid proteome, as well as in plastoglobules, where they play a structural role (Austin *et al.,* 2006; Ytterberg *et al.,* 2006). Over-expression in tomato fruit of one of these proteins originating from *Capsicum annuum*, involved in formation of carotenoid-storing fibrils, has been shown to increase carotenoid content but without fibril formation and to transiently delay thylakoid disappearance in tomato fruit (Simkin *et al.,* 2007). Homologs of the 3 FTSZ proteins from Arabidopsis, which fall into two classes, FTSZ-1 and FTSZ-2, have been identified. These proteins are seen as plastid–located tubulin ancestors. They are involved in plastid division, which is unlikely to occur at this particular fruit development stage. In addition to their stromal location, FTSZ-1 is also present in thylakoids, especially in young chloroplasts,

while FTSZ-2 is also found in the chloroplast envelope. Evidence exists for a functional difference between the two FTSZ classes which may not be limited to plastid division (El-Kafafi *et al.*, 2008). This may explain their presence in chromoplasts.

2.15. Protein import system

Studies on the targeting of nuclear-encoded proteins have defined several pathways to and within the chloroplast (Jarvis, 2008). For accessing the membranes or interior of chloroplast, a Toc/Tic (Translocon at the outer/inner envelope membrane of chloroplast) import machinery is present for the translocation of proteins carrying a transit peptide. Among the Toc complexes, only two of them have been found: Toc64-V and two subunits of Toc75 (Toc75-III and Toc75-V). The latter two proteins were the only Toc75 homologs to be identified at the protein level. Toc75-III is universally expressed in all plant tissues and is therefore believed to be the main import pore of the Toc complex (Vothknecht and Soll, 2005). It is totally embedded in the membrane and functions as a channel through which proteins cross the outer membrane. Toc64 is an accessory member of the Toc complex which serves as docking and guidance for facilitating access to the translocation machinery. Notably, core components such as TOC159 and Toc34, characterized as precursor protein receptors, are absent. Contrary to Toc, many of the proteins proposed to be components of the Tic system by Jarvis (2008) or predicted by Kalanon and McFadden (2008) are present in the chromoplast proteome described in this study (**Fig. 8**). In addition, several proteins of the chaperonin-associated machinery, including an Hsp70 group an Hsp93 and two Cpn60 proteins (Cpn60A and Cpn60B) have been identified. A signal peptide peptidase, SPP, has also been identified. Internal trafficking for transport through the thylakoid to the lumen is mediated by several mechanisms: Sec-, SRP- , Tat-dependent and spontaneous. None of the proteins involved in these pathways was identified, probably as a result of the loss of thylakoid structure. Nevertheless, a number of proteins known to be translocated to the lumen (Klösgen *et al.*, 2004) are present (**Fig.8**).

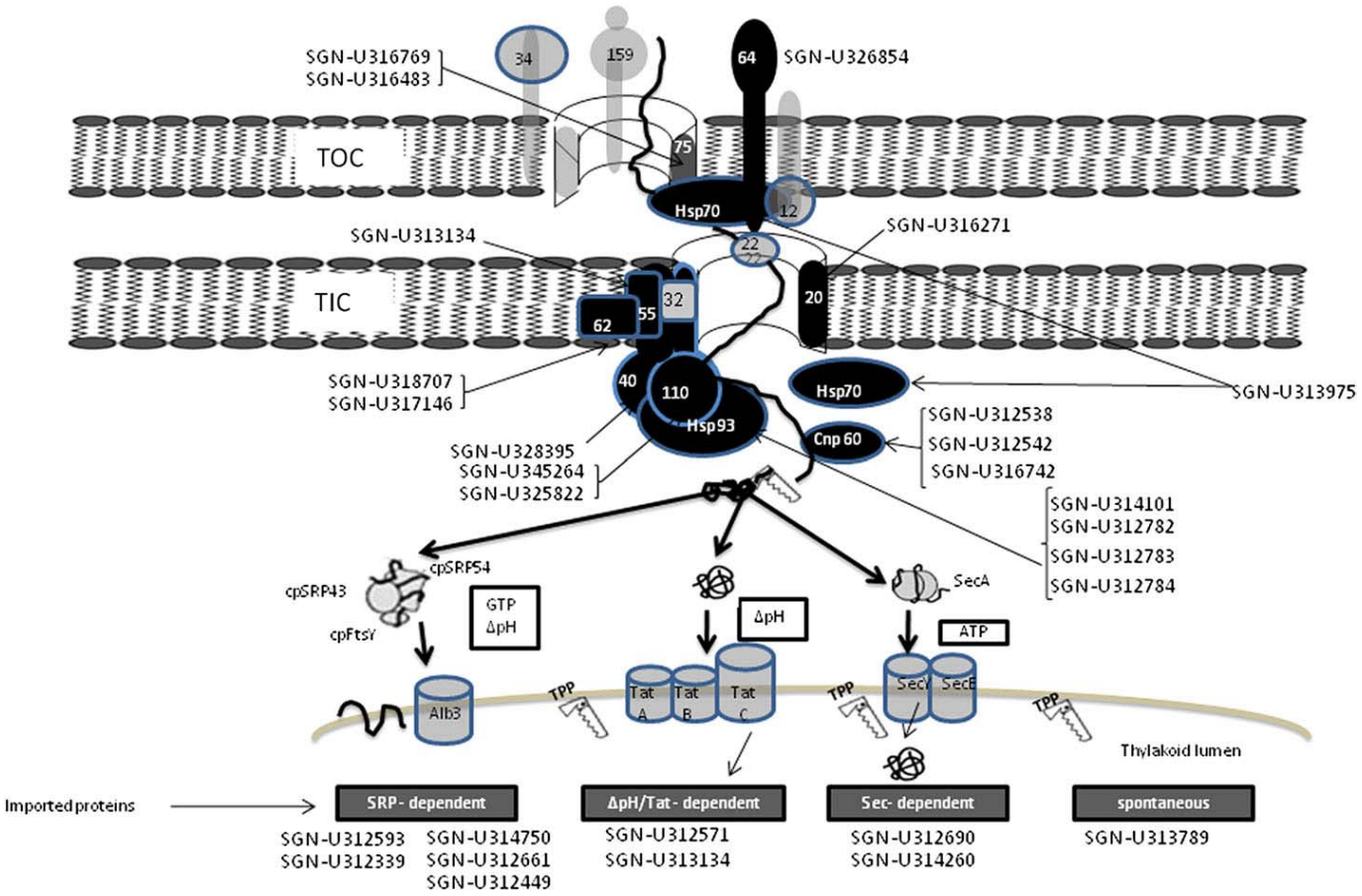


Figure 8: Schematic representation of the plastidial protein import system with mention of proteins encountered in the tomato chromoplast proteome. Note the presence of most of the Tic proteins and the absence of most of the Toc proteins and of all thylakoid import machinery. However, a number of proteins known to be transferred to the lumen by the thylakoid import system are present. Proteins are represented by their unigene SGN code.

The Chloroplast Envelope Quinone Oxidorectase CeQORH has been mentioned as being imported through a non-canonical signal peptide transport by Miras *et al.*, (2002) and Nada and Soll (2004). Intracellular vesicular transport has been observed in chloroplasts by electron microscopy and the use of effectors of vesicle formation (Westphal *et al.*, 2001). Based on predictions made by bioinformatic analysis of the Arabidopsis genome, Andersson and Sandelius (2004) underlined the likely presence in chromoplasts of 33 Arabidopsis homologs of yeast vesicular trafficking components, among which five were detected in the tomato chromoplast proteome (listed in "Vesicular transport" in **Table 2**).

3. Conclusions

The present study reveals a number of important characteristics of the non-photosynthetic plastid, the tomato chromoplast. We report a total of around 1000 chromoplast proteins in tomato. Whilst the predicted size of the plastid proteome of Arabidopsis varies between 1900 - 2500 proteins (Abdallah *et al.,* 2000), 2700 proteins (Millar *et al.,* 2006) and 3800 proteins (Kleffman *et al.,* 2004), the actual number of proteins reported is 1280 (Zybailov *et al.,* 2008). In a study of the pepper chromoplastic proteome, a total of 151 proteins were recorded (Siddique *et al.,* 2006). Anyway, it is clear that the list of plastidial proteins does not represent the whole predicted proteome of the organelle, although uncertainties in the predictions do not allow us to determine the precise coverage of the total proteome. There are several reasons for the limited coverage of the plastidial proteome observed for the tomato chromoplast as well as for other plastids. The number of proteins probably varies according to the development stage of the plastids and environmental conditions. Also, the extraction procedures employed do not yield all the membrane-embedded proteins and low-level soluble proteins. Finally, many proteins are probably present at levels that cannot be detected by the current technologies of separation and sequencing, although the modern Qtrap technology used here can generally detect femtomole levels (10^{-15}). Another consideration that must be taken into account is that proteomic data, similarly to transcriptomic data, are not necessarily indicative of actual metabolic or regulatory activities. Parallel enzymological, metabolomic and fluxomic studies are necessary to fully assess the metabolic activity of the organelle. Nevertheless, proteomic data does give useful information for genome annotation and subcellular localization of proteins. Furthermore, when a whole set of proteins of a specific metabolic pathway is identified, proteomic analysis can give relevant biological information. Out of 325 thylakoid proteins described by various authors in chloroplasts (Peltier *et al.,* 2002, 2004; Giacomelli *et al.,* 2006; Rutschow *et al.,* 2008) we have found 119 in our fractions. These proteins are not involved in a specific pathway; but take part in a variety of processes such as protein degradation, photosynthesis, hormone metabolism etc. Out of these, 23 were found in plastoglobuli (Vidi *et al.,* 2006; Ytterberg *et al.,* 2006) with 14 involved in photosynthesis. Another interesting characteristic of the chromoplast is the total absence of the thylakoid protein transport machinery. An additional observation is the low number of proteins involved in photosynthesis, with only 22% and 39% of the proteins of PSI and PSII respectively. This is related to the absence of chlorophyll and photosynthetic activity associated with the

presence in the chromoplast of active chlorophyll catabolism and autophagy of photosynthetic proteins. On the other hand, the presence of all the Calvin cycle proteins is striking, including all Rubisco subunits and other proteins required for activity. This could be related to the recycling of CO_2 produced by the oxidative pentose phosphate pathway. Another major feature is the capacity for lipid biosynthesis, which is attested by the presence of all the proteins involved in the synthesis of 3-oxoacyl-ACP, the precursor of fatty acids, including all acetyl-CoA-carboxylase monomers. In conclusion, the chromoplast proteome analysis carried out in the present study allows us to gain new insights into the complexity of the functioning of this particular organelle.

Refrences

Abdallah F, Salamini F, Leister D. 2000. A prediction of the size and evolutionary origin of the proteome of chloroplasts of *Arabidopsis. Trends in Plant Science* **5,** 141-142.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *Journal of Molecular Biology* **215,** 403-410.

Andersson MX, Sandelius AS. 2004. A chloroplast-localized vesicular transport system: a bio-informatics approach. *BMC Genomics* **5,** 40, doi:10.1186/1471-2164-5-40

Andersson MX, Goksor M, Sandalius AS. 2007. Optical manipulation reveals strong attracting forces at membrane contact sites between endoplasmic reticulum and chloroplasts. *Journal of Biological Chemistry* **282,** 1170-1174.

Andon NL, Hollingw orth S, Ko ller A, Greenland AJ, Yates 3rd Jr, Haynes PA. 2002. Proteomic characterization of wheat amyloplasts using identification of proteins by tandem mass spectrometry. *Proteomics* **2,** 1156-1168.

Austin II JR, Frost E, Vidi PA, Kessler F, Staehelin LA. 2006. Plastoglobules are lipoprotein subcompartments of the chloroplast that are permanently coupled to thylakoid membranes and contain biosynthetic enzymes. *The Plant Cell* **18,** 1693-1703.

Awai K, X u C, Tamot B, Benning C. 2006. A phosphatidic acid-binding protein of the chloroplast inner envelope membrane involved in lipid trafficking. *Proceedings of the National Academy of Sciences, USA* **103,** 10817-10822.

Baginsky S, Gruissem W. 2004. Chloroplast proteomics: potentials and challenges. *Journal of Experimental Botany* **55,** 1213-1220.

Baginsky S, Siddique A, Gruissem W. 2004. Proteome analysis of tobacco bright yellow-2 (BY-2) cell culture plastids as a model for undifferentiated heterotrophic plastids. *Journal of Proteome Research* **3,** 1128-1137.

Balmer Y, Vensel WH, DuPont FM, Buchanan BB, Hurkman WJ. 2006. Proteome of amyloplasts isolated from developing wheat endosperm presents evidence of broad metabolic capability. *Journal of Experimental Botany* **57,** 1591-1602.

Bedhomme M, Ho ffmann M, McCarthy EA, Gambonnet B, Moran RG, Rébeillé F, Ravanel S. 2005**.** Folate metabolism in plants: an Arabidopsis homolog of the mammalian mitochondrial folate transporter mediates folate import into chloroplasts. *Journal of Biological Chemistry* **280,** 34823-34831.

Birtic S, Ginies C, Causse M, Renard CGC, Page D. 2009. Changes in volatiles and glycosides during fruit maturation of two contrasted tomato (*Solanum lycopersicum*) lines. *Journal of Agricultural and Food Chemistry* **57**, 591–598

Bouvier F, Backhaus A, Camara B. 1998. Induction and control of chromoplast-specific carotenoid genes by oxidative stress. *Journal of Biological Chemistry* **46,** 30651-30659.

Bouyssié D, Peredo AG, Mouton E, Albigot E, Roussel D, Ortega N, Cayrol C, Burlet - Schiltz O, Girard JP, Monsarrat B. 2007. Mascot file parsing and quantification (MFPaQ), a new software to parse, validate, and quantify proteomics data generated by ICAT and SILAC mass spectrometric analyses: Application to the proteomics study of membrane proteins from primary human endothelial cells. *Molecular & Cell Proteomics* **6,** 1621-1637.

Bravdo BM, Palgi A, Lurie S. 1977. Changing ribulose diphosphate carboxylase/oxygenase activity in ripening tomato fruit. *Plant Physiology* **60,** 309-312.

Briat JF, Gigot C, Laulhère J, Mache R. 1982. Visualization of a spinach plastid transcriptionally active DNA-protein complex in a highly condensed structure. *Plant Physiology* **69,** 1205-1212.

Campbell DA, Cock shutt AM, Porankiewicz-Asplund J. 2003. Analysing photosynthetic complexes in uncharacterized species or mixed microalgal communities using global antibodies. *Physiologia Plantarum* **119,** 322-327.

Chen G, Hackett R, Walker D, Taylor A, Lin Z, Grierson D. 2004. Identification of a specific isoform of tomato lipoxygenase (TomloxC) involved in the generation of fatty acid-derived flavor compounds. *Plant Physiology* **136,** 2641-2651.

Chew O, Wheland J, Millar AH. 2003. Molecular definition of the ascorbate-glutathione cycle in Arabidopsis mitochondria reveals dual targeting of antioxidant defenses in plants. *Journal of Biological Chemistry* **278,** 46869-46877.

Delker C, Zolman BK, Miersch O, Wasternack C. 2007. Jasmonate biosynthesis in *Arabidopsis thaliana* requires peroxisomal β-oxidation enzymes- Additional proof by properties of *pex6* and *aim1*. *Phytochemistry* **68,** 1642-1650.

De Santis-Maciossek G, Kofer W, Bock A, Schoch S, Maier RM, Wanner G, Rüdiger W, Koop HR, Herrmann RG. 1999. Targeted disruption of the plastid RNA polymerase genes rpoA, B and C1: molecular biology, biochemistry and ultrastructure. *The Plant Journal* **18,** 477-489.

Deng XW, Gruissem W. 1987. Control of plastid gene expression during development: the limited role of transcriptional regulation. *Cell* **49,** 379-387.

Drescher A, Ruf S, Calsa Jr T, Carrer H, Bock R. 2000. The two largest chloroplast genome-encoded open reading frames of higher plants are essential genes. *The Plant Journal* **22,** 97-104.

Edelman, M, Mattoo, AK. 2008. D1-protein dynamics in photosystem II: the lingering enigma. *Photosynthesis Research* **98**, 609-620.

El-Kafafi E, Karamoko M, Pignot-Paintrand I, Grunwald D, Mandaron P, Lerbs-Mache S, Falconet D. 2008. Developmentally regulated association of plastid division protein FtsZ1 with thylakoid membranes in *Arabidopsis thaliana*. *Biochemical Journal* **409,** 87–94.

Emanuelsson O, Nielsen H, Brunak S, von Heijne G. 2000. Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. *Journal of Molecular Biology* **300**, 1005-1016.

Faurobert M, Mihr C, Bertin N, Pawlowski T, Negroni L, Sommerer N, Causse M. 2007. Major proteome variations associated with cherry tomato pericarp development and ripening. *Plant Physiology* **143,** 1327-1347.

Ferro M, Salvi D, Brugière S, Miras S, Kowalski S, Louwagie M, Garin J, Joyard J,

Rolland N. 2003. Proteomics of the chloroplast enveloppe membranes from *Arabidopsis thaliana*. *Molecular & Cell Proteomics* **2,** 325-345.

Friedrich T, Steinmüller K, Weiss H . 1995. The proton-pumping respiratory complex I of bacteria and mitochondria and its homologue in chloroplasts. *FEBS Letters* **367,** 107-111.

Foyer C, Noctor G. 2003. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiologia Plantarum* **119,** 355–364.

Fraser PD, Kiano JW, Truesdale MR, Schuch W, Bramley PM. 1999. Phytoene synthase-2 enzyme activity in tomato does not contribute to carotenoid synythesis in ripening fruit. *Plant Molecular Biology* **40,** 687-698.

Fraser PD, Pinto ME S, Holloway DE, Bramley PM. 2000. Application of high-performance liquid chromatography with photodiode array detection to the metabolic profiling of plant isoprenoids. *The Plant Journal* **24,** 551-558.

Giacomelli L, Rudella A, van Wijk KJ. 2006. High light response of the thylakoid proteome in Arabidopsis wild type and the ascorbate-deficient mutant vtc2-2. A comparative proteomic study. *Plant Physiology* **141,** 685-701.

Giacomelli L Masi A, Ripoll DR, Lee MJ, va n Wijk KJ. 2007. *Arabidopsis thaliana* deficient in two chloroplast ascorbate peroxidases shows accelerated light-induced necrosis when levels of cellular ascorbate are low. *Plant Molecular Biology* **65,** 627-644.

Giovannoni J. 2001. Molecular biology of fruit maturation and ripening. *Annual Review of Plant Physiology and Plant Molecular Biology* **52,** 725-749.

Griffiths A, Barry C, Alpuche-Solis AG, Grierson D. 1999. Ethylene and developmental signals regulate expression of lipoxygenase genes during tomto fruit ripening. *Journal of Experimental Botany* **50**, 793-798.

Harlingue (d') A, Camara B. 1985. Plastid enzymes of terpenoid biosynthesis. Purification and characterization of gamma-tocopherol methyltransferase from Capsicum chromoplasts. *Journal of Biological Chemistry* **260:** 15200-15203.

Heazlewood JL, Verboom RE, Tonti- Filippini J, Small I, Millar AH . 2007. SUBA: the Arabidopsis Subcellular Database. *Nucleic Acids Research* **35**, D213-D218.

Herrmann KM , Weaver LM . 1999. The shikimate pathway. *Annual Review of Plant Physiology and Plant Molecular Biology* **50,** 473-503.

Hesse H, Hoefgen R. 2003. Molecular aspects of methionine biosynthesis. *Trends in Plant Science* **8:** 259-262.

Hudák J, Galova E, Zemanova L. 2005. Plastid morphogenesis. In M Pessarakli, ed, Handbook of Photosynthesis, Ed 2, CRC Press, Boca Raton, pp 221-246I

Hunt CM, Hardison RC, Boyer CD. 1986. Restriction enzyme analysis of tomato chloroplast and chromoplast DNA. *Plant Physiology* **82,** 1145-1147.

Jarvis P. 2008. Targeting of nucleus-encoded proteins to chloroplasts in plants. *New Phytologist* **179,** 257-285.

Julliard JH, Douce R. 1991. Biosynthesis of the thiazole moiety of thiamine (vitamin B1) in higher plant chloroplasts. *Proceedings of the National Academy of Sciences, USA* **88,** 2042- 2045.

Kahlau S, Aspinall S, Gray JC, Bock R . 2006. Sequence of the tomato chloroplast DNA and evolutionary comparison of solanaceous plastid genomes. *Journal of Molecular Evolution* **63,** 194-207.

Kahlau S, Bock R. 2008. Plastid transcriptomics and translatomics of tomato fruit development and chloroplast-to-chromoplast differentiation: chromoplast gene expression largely serves the production of a single protein. *The Plant Cell* **20,** 856-874.

Kalanon M, McFadden I. 2008. The chloroplast protein translocation complexes of *Chlamydomonas reihardtii*: a bio-informatic comparison of Toc and Tic components in plants, green algae and red algae. *Genetics* **179,** 95-112.

Keresztes A, Schróth A. 1979. Light and electron microscopic investigation of *in vitro* starch synthesis in chromoplasts. *Cytobios* **26,** 185-191.

Kleffmann T, Russenberger D, von Zychlinski A, Christopher W, Sjölander K, Gruissem W, Baginsky S. 2004. The *Arabidopsis thaliana* chloroplast proteome reveals pathway abundance and novel functions. *Current Biology* **14,** 354-362.

Kleffmann T, Hirsch-Hoffmann M, Gruissem W, Baginsky S. 2006. plprot: a comprehensive proteome database for different plastid types. *Plant Cell Physiology*. 47, 432-6.

Klösgen RB, Molik S, Frielingsdorf S, Gutensohn M, Jakob M, Marques JP, Hou B. 2004. Protein transport across the thylakoid membrane. *Endocytobiosis and Cell Research* **15,** 518- 526.

Kroll D, Meierho ff K, Bechto ld N, Kinoshita M, Westpha l S, Voth k necht UC, Soll J, Westhoff P. 2001. *VIPP1,* a nuclear gene of Arabidopsis thaliana essential for thylakoid membrane formation. *Proceedings of the National Academy of Sciences, USA* **98,** 4238-4242.

Kruger NJ, von Schaew en A. 2003. The oxidative pentose phosphate pathway: structure and organisation. *Current Opinion in Plant Biology* **6,** 236-246.

Kwok EY, Hanson MR. 2004. Stromules and the dynamic nature of plastid morphology. *Journal of Microscopy* **214,** 124-137.

Laizet Y, Pontier D, Mache R, Ku ntz M. 2004. Subfamily organization and phylogenic origin of genes encoding plastid lipid-associated proteins of the fibrillin type. *Journal of Genome Science and Technology* **3,** 19-28.

Lelièvre JM, Latché A, Jones B, Bouzayen M, Pech JC. 1997. Ethylene and fruit ripening. *Physiologia Plantarum* **101,** 727-739.

Livne A, Gepste in S. 1988. Abundance of the major chloroplast polypeptides during development and ripening of tomato fruits. *Plant Physiology* **87,** 239-242.

Martinez-Madrid MC, Serrano M, Riquelme F, Romojaro F. 1996. Polyamines, abscisic acid and ethylene production in tomato fruit. *Phytochemistry* **43,** 323-326

Marano MR, Carrillo N. 1992. Constitutive transcription and stable RNA accumulation in plastids during the conversion of chloroplasts to chromoplasts in ripening tomato fruits. *Plant Physiology* **100,** 1103-1113.

Marti MC, Camejo D, Olmos E, Sandalio LM, Fernandez-Garcia N, Jimenez A, Sevilla F. 2009. Characterization and changes in the antioxidant system of chloroplasts and chromoplasts isolated from green and mature pepper fruits. *Plant Biology* 11:613-624.

Mattoo AK, Hoffma n-Falk H, Marder JB, Edelman M. 1984. Regulation of protein metabolism: Coupling of photosynthetic electron transport to in vivo degradation of the rapidly metabolized 32-kilodalton protein of the chloroplast membranes. *Proceedings of the National Academy of Sciences, USA* **81,** 1380–1384.

Millar AH, Whelan J, Small I. 2006. Recent surprises in protein targeting to mitochondria and plastids. *Current Opinion in Plant Biology* **9,** 610-615.

Miras S, Salvi D, Ferro M, Grunwald D, Garin J, Joyard J, Rolland N. 2002. Non-canonical transit peptide for import into the choloroplast. *Journal of Biological Chemistry* **227,** 4770- 4778.

Moore S., Vrebalov J., Payton P, Giovannoni J. 2002. Use of genomics tools to isolate key ripening genes and analyse fruit maturation in tomato. *Journal of Experimental Botany* **53,** 2023- 2030.

Morstadt L, Gräber P, Pascalis L, Klein ig H, Speth V, Beyer P. 2002. Chemiosmotic ATP synthesis in photosynthetically inactive chromoplasts from *Narcissus pseudonarcissus* L. linked to a redox pathway potentially also involved in carotene desaturation. *Planta* **215,** 134-140.

Nada M, Soll J. 2004. Inner envelope protein 32 is imported into chloroplasts by a novel pathway. *Journal of Cell Science* **117,** 3975-3982.

Ouyang J, Shao X, Li J. 2000. Indole-3-glycerol phosphate, a branchpoint of indole-3-acetic acid biosynthesis from the tryptophan biosynthetic pathway in *Arabidopsis thaliana*. *The Plant Journal* **24**, 327-333.

Peeters N, Small I. 2001. Dual targeting to mitochondria and chloroplasts. *Biochimica et Biophysica Acta* **1541,** 54-63.

Peltier JB, Emanuelsso n O, Ka lume DE, Yt terberg J, Friso G, Rudella A, L iberles DA, Soderberg L, Roepstorff P, von Heijne G, van Wijk KJ. 2002. Central functions of the luminal and peripheral thylakoid proteome of Arabidopsis determined by experimentation and genome-wide prediction. *The Plant Cell* **14,** 211-236.

Peltier JB, Ytterberg J , Sun Q, van Wijk KJ. 2004. New functions of the thylakoid membrane proteome of Arabidopsis thaliana revealed by a simple, fast, and versatile fractionation strategy. *Journal of Biological Chemistry* **279,** 49367-49383.

Peltier JB, Cai Y, Sun O, Z abrouskov V, Giacomelli L, Rudella A, Ytterberg AJ, Rutschow H, an Wijk KJ (2006) The oligomeric stromal proteome of *Arabidopsis thaliana* chloroplasts. *Molecular & Cell Proteomics* **5,** 114-133.

Piechulla B , Glick RE , Bahl H, Melis A, Gruissem W. 1987. Changes in photosynthetic capacity and photosynthetic protein pattern during tomato fruit ripening. *Plant Physiology* **84,** 911-917.

Pruzinska A, Tanner G, Aubry S, Anders I, Moser S, Müller T, Ongania KH, Kräutler B, Youn JY, Liljegren SJ, Hörtensteiner S 2005. Chlorophyll breakdown in senescent Arabidopsis leaves. Characterization of chlorophyll catabolites and of chlorophyll catabolic enzymes involved in the degreening reaction. *Plant Physiology* **139,** 52-63.

Railton ID, Fellows B, West CA. 1984. *Ent*-Kaurene synthesis in chloroplasts from higher plants. *Phytochemistry* **23,** 1261-1267.

Ravanel S, Block MA, Rippert P, Jabrin S, Curien G, Rébeillé F, Douce R. 2004. Methionine metabolism in plants. Chloroplasts are autonomous for de novo methionine synthesis and can import S-adenosylmethionine from the cytosol. *Journal of Biological Chemistry* **279,** 22548-22557.

Ren G, An K, Liao Y, Zhou X, Cao Y, Zhao H, Ge X, Kuai B. 2007. Identification of a novel chloroplast protein AtNYE1 regulating chlorophyll degradation during leaf senescence in Arabidopsis. *Plant Physiology* **144,** 1429-1441.

Richards CM, Hinman SB, Boyer CD, Hardison RC. 1991. Survey of plastid RNA abundance during tomato fruit ripening: the amounts of RNA from the ORF2280 region increases in chromoplasts. *Plant Molecular Biology* **17,** 1179-1188.

Roje S. 2007. Vitamin B biosynthesis in plants. *Phytochemistry* **68,** 1904-1921.

Rolland F, Sheen J. 2005. Sugar sensing and signalling networks in plants. *Biochemical Society Transactions* **33,** 269-271.

Ronen G, Cohen M, Zamir D, Hirschberg J. 1999. Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon cyclase is down-regulated during ripening and is elevated in the mutant delta. *The Plant Journal* **17,** 341-351.

Rutschow H, Ytterberg AJ, Friso G, Nilsson R, van Wijk KJ. 2008. Quantitative proteomics of a chloroplast *SRP54* sorting mutant and its genetic interaction with *CLPC1* in Arabidopsis. *Plant Physiology* **148**, 156-175.

Schelbert S, Aubry S, Burla B, Agne B, Kessler F, Krupinska K, Hörtensteiner S. 2009. Pheophytin pheophorbide hydrolase (pheophytinase) is involved in chlorophyll breakdown during leaf senescence in Arabidopsis. *The Plant Cell* **21,** 767-785.

Schubert M, Petersson UA, Haas BJ, Funk C, Schroder WP, Kieselbach T. 2002. Proteome map of the chloroplast lumen of *Arabidopsis thaliana*. *Journal of Biological Chemistry* **277,** 8354-8365.

Schwender J, Gof fman F, Ohlr ogge JB, Shachar-Hill Y. 2004. Rubisco without the Calvin cycle improves the carbon efficiency of developing green seeds. *Nature* **432,** 779-782.

Shahbazi M, Gilbert M, Labouré AM, Ku ntz M. 2007. Dual role of the plastid terminal oxidase in tomato. *Plant Physiology* **145,** 691-702.

Siddique MA, Grossmann J, Gruissem W, Baginsky S. 2006. Proteome analysis of bell pepper (*Capsicum annuum* L.) chromoplasts. *Plant & Cell Physiology* **47,** 1663-1673.

Simkin AJ, Gaffé J, Alacaraz JP, Carde JP, Bramley PM, Fraser PD, Kuntz M. 2007. Fibrillin influence on plastid ultrastructure and pigment content in tomato fruit. *Phytochemistry* **68,** 1545-1556.

Soll J. 2002. Protein import into chloroplasts. *Current Opinion in Plant Biology* **5,** 529-535.

Speirs J, Lee E, Holt K, Yong-Duk K, Steele Scott N, Loveys B, Schuch W. 1998. Genetic manipulation of alcohol dehydrogenase levels in ripening tomato fruit affects the balance of some flavor aldehydes and alcohols. *Plant Physiology* **117,** 1047-1058.

Stepanova AN, Hoyt JM, Hamilton AA, Alonso JM. 2005. A link between ethylene and auxin uncovered by the characterization of two root-specific ethylene-insensitive mutants in Arabidopsis. *The Plant Cell* **17,** 2230-2242.

Strand A, Asami T, Alonso J, Ecker JR, Chory J. 2003. Chloroplast to nucleus communication triggered by accumulation of Mg-protoporphyrinIX. *Nature* **421,** 79-83.

Sun Q, Zybailov B, Majeran W, Friso G, Olinares PD, van Wijk KJ. 2008. PPDB, the Plant Proteomics Database at Cornell. *Nucleic Acids Research* **37**, D979-D974

Surpin M, Larkin RM, Chory J. 2002. Signal transduction between the chloroplast and the nucleus. *The Plant Cell* **14,** S327-S338.

Terry MJ , Lagarias JC. 1991. Holophytochrome assembly. Coupled assay for phytochromobilin synthase *in organello*. *Journal of Biological Chemistry* **266,** 22215-22221.

Tetlow IJ, Bow sher GG, Emes MJ. 2003. Biochemical properties and enzymatic capacities of chromoplasts isolated from wild buttercup (*Ranunculus acris* L.). *Plant Sciences* **165**, 383- 394.

Thimm O, Blasing O, Gibon Y, Nagel A, Meyer S, Kruger P, Selbig J, Muller LA, Rhee SY, Stitt M. 2004. MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *The Plant Journal* **37,** 914-939.

Thom E, Möhlmann T, Quick WP, Camara B, Neuhaus H-E. 1998. Sweet pepper plastids: enzymatic equipment, characterization of the plastidic oxidative pentose-phosphate pathway, and transport of phosphorylated intermediates across the envelope membrane. *Planta* 204, 226-233.

Thomas H, Huang L, Young M, Ougham H. 2009. Evolution of plant senescence. *BMC Evolutionary Biology* **9,** 163 doi:10.1186/1471-2148-9-163.

Vidi PA, Kanwischer M, Baginski S, Austin JA, Csucs G, Dörmann P, Kessler F, Bréhélin C. 2006. Tocopherol cyclase (VTE1) localization and vitamin E accumulation in chloroplasts plastoglobule lipoprotein particles. *Journal of Biological Chemistry* **281,** 11225-11234.

Vothknecht UC, Soll J. 2005. Chloroplast membrane transport: interplay of procaryotic and eucaryotic traits. *Gene* **354,** 99-109.

von Zychlinski A , Kleffmann T, Krishnamurthy N, Sjölander K, Baginsky S, Gruissem W. 2005. Proteome analysis of the rice etioplast: metabolic and regulatory networks and novel protein functions. *Molecular & Cell Proteomics* **4,** 1072-1084.

Wada S, Ishida H, Izumi M, Yoshimoto K, Ohsumi Y, Mae T, Makino A. 2009. Autophagy plays a role in chloroplast degradation during senescence in individually darkened leaves. *Plant Physiology* **149,** 885-893.

Westphal S, Soll J, Vothknecht UC. 2001. A vesicle transport system inside chloroplasts. *FEBS Letters* **506,** 257-261.

Whatley JM, McLean B, Juniper BE. 1991. Continuity of chloroplast and endoplasmic reticulum membranes in *Phaseolus vulgaris. New Phytologist* **117,** 209-217.

Wilm M, Shevchenko A, Houthaeve T, Breit S, Schweigerer L, Fotsis T, Mann M .1996. Femtomole sequencing of proteins from polyacrylamide gels by nano-electrospray mass spectrometry. *Nature* **379,** 466-469.

Wiese A, Gröner F, Sonnewald U, Deppner H, Lerchl J, Hebbeker U, Flügge UI, Weber A. 1999. Spinach hexokinase I is located in the outer envelope membrane of plastids. *FEBS Letters* **461,** 13-18.

Yamaguchi K, Subramanian AR . 2003. Proteomic identification of all plastid-specific ribosomal proteins in higher plant chloroplast 30S ribosomal subunit PSRP-2 (U1A-type domains), PSRP-3a/b (ycf65 homologue) and PSRP-4 (Thx homologue). *European Journal of Biochemistry* 270, 190–205.

Ytterberg AJ, Peltier JB, van Wijk KJ. 2006. Protein profiling of plastoglobules in chloroplasts and chromoplasts. A surprising site for differential accumulation of metabolic enzymes. *Plant Physiology* **140,** 984-997.

Yu TS, Kofler H, Hä user RE, Hille D, Flügge UI, Z eeman SC, Smith AM, Kossmann J, Lloyd J, Ri tte G, Steu p M, Lue WL, Chen J, Weber A. 2001. The Arabidopsis sex1 mutant is defective in the R1 protein, a general regulator of starch degradation in plants, and not in the chloroplast hexose transporter. *The Plant Cell* **13,** 1907-1918.

Zybailov B, Rutschow H, Friso G, Rudella A, Emanuelss on O, Sun Q, van Wij k KJ. 2008. Sorting signals, N-terminal modifications and abundance of the chloroplast proteome. *PLoS One* **3,** e1994.

Zybailov B, Friso G, Kim J, Rudella A, Rami rez Ro driguez V, Asakura Y, Sun Q, van Wijk KJ. 2009. Large scale comparative proteomics of a chloroplast Clp protease mutant reveals folding stress, altered protein homeostasis and feedback regulation of metabolism. *Molecular & Cell Proteomics* **8**, 1789-1810.

Table 2: **Selected proteins identified in the tomato chromoplast proteome and discussed in the text.** Classification has been made essentially according to MapMan with minor adjustments as mentioned in "Material and Methods".

Name and functional information	*SGN code*
1-PHOTOSYNTHESIS AND CALVIN	
Photosystem (PS)II	
chlorophyll A-B binding prot1	U313211, U313204, U313212 , U313213, U313214
light-harvesting Chl a/b-binding (LHCB6)	U312339
LHCB2.1	U312436, U312438
LHCB5	U312449
LHCB4	U312661
LHCB3	U314750
thylakoid lumenal 29.8 kDa protein	U324760, U318255
PS II family protein	U319106
O2-evolving enhancer prot 2-1	U312571, U312572
nonphotochemical quenching4	U312967
oxygen-evolving complex	U325341
PSII subunit O-2	U312871, U312532, U312531
PS II reaction centerPsbP fam prot	U323580,U317040
CP43 subunit of the PS II	U343039
PSI	
LHCA3	U312843
LHCA4	U312593, U317042
PS I subunit D-2	U312640
PS I subunit F	U314260
PS I subunit E-2	U313447
Cytochrome B6/f	
photosynthetic electron transfer C	U312858
cytochrome f apoprotein	U342407
cytochrome b(6) subunit	U331670
Lightreactions(LR) .ATP synthase	
ATP synthase γ chain 1	U313245
ATP synthase δ chain	U313693
ATP synthase family	U313789
ATPase α subunit	U329173, U323749
ATPase F subunit	U340510
LR. electron carrier (ox/red)	
DNA-damage-repair/toleration protein	U312690
NADPH dehydrogenase/ oxidoreductase	U314955
Autophagy	
senescence-associated gene(SEN1)	U316277
Tetrapyrolle synthesis	
glutamyl-tRNA(Gln) amidotransferase	U323134
ferrochelatase I	U316403
glutamate-1-semialdeh 2,1-aminomutase	U315915
sirohydrochlorin ferrochelatase	U323001
porphobilinogen synthase	U312668
hydroxymethylbilane synthase	U315567
uroporphyrinogen decarboxylase	U315267
coproporphyrinogen oxidase	U315993
protoporphyrinogen oxidase	U326999
Chlorophyll breakdown	
pheophytinase	U317890
phaeophorbide a oxygenase	U313134
stay-green protein 1	U316068
Calvin cycle : RuBisCO and RuBisCO related	
large subunit of RuBisCO gi|89280643,U344009,U346314	
RuBisCO small subunit 1A	U314254, U314262
RuBisCO small subunit 3B	U314700, U314701, U314722 U338973
RuBisCO activase	U312543, U312544
RuBisCo large subunit N-methyltransferase	U326460
chaperonin 60α	U312538, U312542
chaperonin 60β	U316742
Calvin cycle : others	
ribulose-PH 3-epimerase	U313308
phosphoribulokinase	U312791
phosphoglycerate kinase	U313176
glycerald-3-PH DH β	U312802, U312461,U312804
triose-PH isomerase	U313729
fructose-bisPH aldolase	U314787, U314788, U312344 U312608, U312609
fructose-1,6-bisphosphatase	U316424
OPP pathway	
ribose 5-phosphate isomerase	U315528
transketolase, putative	U312320, U312319, U312322 U323721
transaldolase	U315742, U315064
2-CARBOHYDRATE METABOLISM	
glucose 6-phosphate transporter	U330538
triose phosphate translocator	U312460
starch synthase I	U318293
ADP-glucose pyrophosphorylase	U317866
1,4-α-glucan branching enzyme	U312423, U312427
α-amylase3	U317456 ; U326232, U326817
β-amylase 3	U313315
glucan phosphorylase	U316416, U316417, U325849, U345057
phosphoglucan, water dikinase	U328612
disproportionating enz 1	U322816, U333138, U342143
disproportionating enz 2	U327405
isoamylase3	U328875, U333011
starch excess 1	U315116
starch excess 4	U317732
3-LIPID SYNTHESIS AND METABOLISM	
Fatty acid synthesis	
pyruvate dehydrogenaseE1α	U313753
pyruvate dehydrogenaseE1β	U314162
pyruvate dehydrogenaseE2	U317019
biotin carboxyl-carrier prot CAC1	U317261, U317459
biotin carboxylase subunit 2 CAC2	U324109, U327019
acetyl-CoA carboxylase CAC3	U323169, U317741
acetyl-CoA carboxylase βsubunit ACCD	gi|89280644
S-malonyltransferase	U316102
3-ketoacyl-acyl carrier prot synth I	U315474, U315475, U325875
3-ketoacyl-acyl carrier prot synth III	U316868, U335865
3-oxoacyl-ACPreductase	U315110
β-hydroxyacyl-ACP dehydratase	U319205
enoyl-ACP reductase	U321872,U315697
Phospholipid synthesis	
Name and functional Information	*SGN co*
trigalactosyldiacylglycerol2	U321
plastid transcriptionally active4=VIPP1	U317521
Lipid catabolism	
phospholipase D α 1	U316492, U3188
LOXC	U3156
hydroperoxide lyase	U3158
alcohol dehydrogenase2	U3143
4-AMINO ACID METABOLISM	
Shikimate pathway	
3-deoxy-7-phosphoheptulonate synthase	U3192
shikimate 5 DH	U318401, U3219
3-phosphoshikimate 1-carboxyvinyltransf.	U3174
chorismate synthase	U3133
Aromatic aa.tryptophan synthesis.	
anthranilate synthase β subunit. 1	U3306
anthranilate synthase α2	U321504, U3215
anthranilate phosphoribosyltransf	U3219
tryptophan indole-3-glycerol PH synthase	U3182
tryptophan synthase, α subunit	U3175
tryptophan synthase, β subunit	U3172
Methionine synthesis	
homoserine kinase	U3218
threonine synthase	U316421,3305
aspartate semialdehyde DH	U3203
aspartate kinase/ homoserine DH	U3173
cystathionine β-lyase	U3206
Sulfur assimilation	
sulfite oxidase	U3143
ATP sulfurylase 3	U313496, U3134
Ammonia assimilation	
glutamine synthetase	U3145
ferredoxin dep glutamate synth 1	U317540, U3232
nitrate reductase	U3175
5-TERPENOID METABOLISM	
Rohmer (non-mevalonate) pathway	
1-deoxyxylulose 5-PH synthase	U3162

Photorespiration	glucose-6-PH dehydrogenase2 U317444	phosphatidylglycerolphosphate synthase U319207	1-Deoxy-d-xylulose 5-PH reductoisomerase U315
phosphoglycolate phosphatase U314994	glucosamine/galactosamine-6-PH isomerase U315096	**Glycolipid and sulfolipid synthesis**	2CmethylDerythritol 2,4cyclodiPH synt U318
(S)-2-hydroxy-acid oxidase U312724, U333873	U315098,U318386	UDPsulfoquinovose synthase U317216, U317217	2CmethylDerythritol PH cytidyltransferase U319
glycine cleavage system H U312985	phosphogluconate dehydrogenase U316131	UDP-glucose:sterol glucosyltransferase U317386,	4hydroxy3methylbut2en1yl diPH synt U3141
serine hydroxymethyl transferase3 U319359, U319360	6-phosphogluconate DH U318328,U332994	U330998	
Mevalonate pathway	glutathione peroxidase U315143, U315728	short-chain dehydrogenase U326257	tic62 U318707,U3171
acetoacetyl-Coa thiolase 2 U314024	monodehydroascorbate reductase U320487, U345138	**ABA catabolism**	tic55 U3131
isopentenyl-diPH delta-isomerase U315069	dehydroascorbate red uctase U313719, U313537	(+) abscissic 8'hydroxylase U325016	tic40 U3283
Prenyl transferases	peroxiredoxin Q U314061	**Jasmonates synthesis**	tic20 II U3162
geranylgeranyl pyroPH s synthase U326688, U325914	peroxiredoxin type 2 U314448	LOX2 U315633	**Chaperonin associated machinery**
geranylgeranyl reductase U316915,U313450	2-cys peroxiredoxin U314924,U314923	LOX3 U321151	heat shock protein 70-1 U3139
Lycopene biosynthesis	thioredoxin M-type 1 U329463	allene oxide synthase U319339	heat shock protein 70-7 U3157
phytoene synthase 1 U314429	thioredoxin M-type 4 U316173,U318067	allene oxide cyclase U316550	heat shock protein 93-V U312782, U312783, U3127
phytoene desaturase U318137	thioredoxin oprotein2 U319145	**9-SIGNALLING ELEMENTS**	60 kDa chaperonin α subunit U312538, U3125
zeta-carotene desaturase U335523,U316184	thioredoxin reductase U324098	**Glucose signalling**	60 kDa chaperonin β subunit U3167
6-VITAMIN BIOSYNTHESIS	**Redox enzymes**	hexokinase1 U328823, U335794	signal peptide peptidase (SPPA) U3411
Riboflavin (vitamin B2)	catalase U323590, U312411, U323759	**Calcium signaling**	**Proteins translocated to the lumen**
GTP cyclohydrolase II U317027	Cu, Zn superoxide dismutase 2 U315383, U315384	calmodulin-binding heat-shock U327931	LHCP3 U3147
6,7-dimethyl-8-ribityllumazine synthase U322093	Cu/Zn superoxide dismutase 1 U317104	IQ calmodulin-binding U324954	LHCP5 U3124
lumazine-binding family protein U323258	Fe-superoxide dismutase U313819,	calnexin 1 U315861	LHCP6=CP24 U3123
Panthothenate (vitamin B5)	U317645, U319423, U314438	Ca-binding EF hand fam U318939	23 kDa prot U3125
beta-ureidopropionase U321324	NADH-ubiquinone o/r 24 kDa U316255	**10-STRUCTURAL AND BUILDING BLOCKS**	Rieske protein U3131
Folate (vitamin B9)	NADH-ubiquinone o/r 51 kDa U316563	**Plastid lipid associated**	PC=DNA-damage-repair/toleration prot 112 U3126
4-amino-4-desoxychorismate lyase U314873	NADH-ubiquinone o/r 20 kDa U343941	PAP / fibrillin fam protein U341818, U319404,	PS I subunit F U3142
Folate metabolism	NADH-ubiquinone o/r 75 kDa U315458	U321757,U319105, U316479, U316793, U313480	Cfo-II= chloroplastic quinone-o/r U3137
serine hydroxymethyltransferase U315084		FTSZ1-1 U320588, U328370	CeQORH U3141
Tocopherols (vitamin E)	**8-HORMONES**	FTSZ2-2 U324505	**12-VESICULAR TRANSPORT**
methyltransferase U313381	**IAA synthesis**	**11- PROTEIN IMPORT SYSTEM**	COPII U318
γ-tocopherol methyltransferase activity U317964	tryptophan indole-3-glycerol PH synthase U318203	**Toc/Tic**	clathrin heavy chain U321296, U3468
7-REDOX	anthranilate synthaseβ subunit. 1 U330695	tic32-IVb U314839	coatomer β subunit U3153
Ascorbate-glutathione cycle	anthranilate synthase α2 U321504, U321505	tIC110 U345264, U325822	coatomer γ2 subunit U3272
stromal L-ascorbate peroxidase U314092	**ABA synthesis**	toc75-III U316483	
thylakoid L-ascorbate peroxidase U314093	zeaxanthin epoxidase U321035	Toc 75 V U316769	

Chapter III

METABOLIC AND MOLECULAR EVENTS OCCURRING DURING THE BIOGENESIS OF CHROMOPLASTS

Cristina BARSAN [1,2,a], Isabel EGEA[1,2,a], Wanping BIAN [1,2], Eduardo PURGATTO[3], Christian CHERVIN[1,2], Alain LATCHÉ[1,2], Mondher BOUZAYEN[1,2] and Jean-Claude PECH[1,2, a] Participated equally to the work - Submitted to Journal of Botany – September 2010

Abstract

Chromoplasts are non-photosynthetic plastids that accumulate carotenoids. They derive from other plastid forms, mostly chloroplasts. The biochemical events responsible for the inter-conversion of one plastid form into another are poorly documented. However, thanks to transcriptomics and proteomics approaches, novel information is now available. Data of proteomic and biochemical analysis revealed the importance of lipid metabolism and carotenoids biosynthetic activities. The loss of photosynthetic activity was associated with the absence of the chlorophyll biosynthesis branch and the presence of proteins involved in chlorophyll degradation. Surprisingly, the entire set of Calvin cycle and of the oxidative pentose phosphate pathway persisted after the transition from chloroplast to chromoplast. The role of plastoglobulines in the formation and organisation of carotenoid-containing structures and that of the *Or* gene in the control of chromoplastogenesis are reviewed. Finally, using transcriptomic data, an overview is given of the expression pattern of a number of genes encoding plastid-located proteins during tomato fruit ripening.

Introduction

Chromoplasts are non-photosynthetic plastids that accumulate carotenoids and give a bright colour to plant organs such as fruit, flowers, roots and tubers. They derive from chloroplasts such as in ripening fruit [1], but they may also arise from proplastids such as in carrot roots [2] or from amyloplasts such as in saffron flowers [3] or tobacco floral nectaries [4]. Chromoplasts are variable in terms of morphology of the carotenoid-accumulating structures and the type of carotenoids [5,6]. For instance, in tomato, lycopene is the major carotenoid and it accumulates in membrane-shaped structures [7] while in red pepper beta-carotene is prominent and accumulates mostly in large

globules [8]. Reviews specifically dedicated to the biogenesis of chromoplasts have been published [9-11]. Some information can also be found in papers dedicated to plastid differentiation in general [12,13]. Thanks to transcriptomics and proteomics approaches, novel information is now available on the biochemical and molecular aspects of chromoplasts differentiation [14-16]. The present paper will review these novel data and provide a recent view of the metabolic and molecular events occurring during the biogenesis of chromoplasts and conferring specificities to the organelle. Focus will be made on the chloroplast to chromoplast transition.

1. *Chromoplast differentiation is associated with important structural, metabolic and molecular re-orientations*

Important structural changes occur during the chloroplast to chromoplast transition, thylakoid disintegration being the most significant (Figure 1).

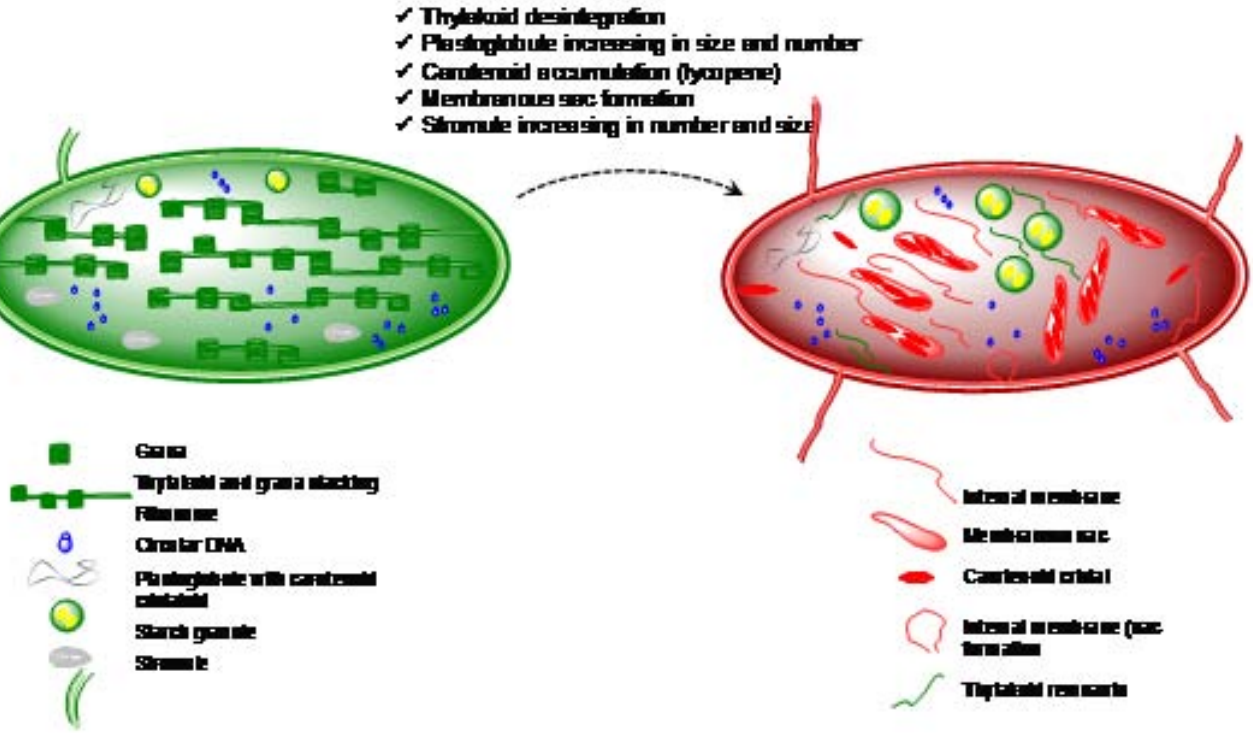


Figure 1: Schematic representation of the main structural changes occurring during the chloroplast-to-chromoplast transition

Early microscopic observations have shown that plastoglobuli increase in size and number during the chloroplast-chromoplast transition [7] and that the internal membrane system is profoundly affected at the level of the grana and inter-grana thylakoids [17]. Stromules (stroma-filled tubules), that are dynamic extensions of the plastid envelope allowing communication between

plastids and other cell compartments like the nucleus [18 are also affected during chromoplastogenesis. A large number of long stromules can be found in mature chromoplasts contrasting with the few small stromules of the chloroplasts in green fruit [19]. It can therefore be assumed that the exchange of metabolites between the network of plastids and between the plastids and the cytosol is increased in the chromoplast as compared to the chloroplast. However, the most visible structural change is the disruption of the thylakoid grana, the disappearance of chlorophyll and the biogenesis of carotenoid-containing bodies. Associated with the structural changes, the toc/tic trans-membrane transport machinery is disintegrated [16,20]. The non canonical signal peptide transport [21] and intracellular vesicular transport [22,23] may represent the most active form of trans-membrane transport into the chromoplast as compared to the chloroplast. Proteins involved in vesicular transport were detected in the tomato chromoplastic proteome [16].

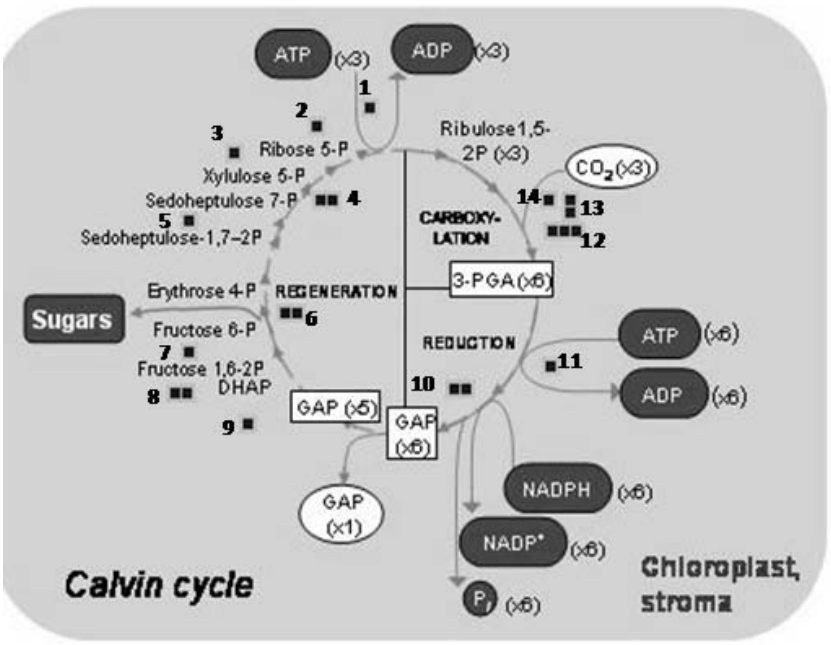
One of the most visible metabolic changes occurring during the chloroplast to chromoplast transition is the loss of chlorophyll and the accumulation of carotenoids [24]. The photosynthetic machinery is strongly disrupted and a reduction in the levels of proteins and mRNAs associated with photosynthesis was observed [25] Also the decrease in photosynthetic capacity during the later stages of tomato fruit development was confirmed by transcriptomic data [26]. However part of the machinery persist in the chromoplast. It has been suggested that it participates in the production of C4 acids, in particular malate a key substrate for respiration during fruit ripening [27]. In the tomato chromoplast proteome, all proteins of the chlorophyll biosynthesis branch are lacking [16]. In the early stages of tomato fruit ripening the fruits are green and the plastids contain low levels of carotenoids that are essentially the same as in green leaves, i.e. mainly β-carotene, lutein, and violaxanthin. At the ‘‘breaker’’ stage of ripening lycopene begins to accumulate and its concentration increases 500-fold in ripe fruits, reaching *ca*. 70 mg/g fresh weight [24]. During the ripening of tomato fruit an up-regulation of the transcription of *Psy* and *Pds*, which encode phytoene synthase and phytoene desaturase, respectively, was reported [28]. One of the main components of the carotenoid-protein complex, a chromoplast-specific 35-kD protein (chrC) purified and characterized from *Cucumis sativus* corollas, was studied and it was shown that its steady-state level increased in parallel with flower development and carotenoid accumulation, peaking in mature flowers [29] In tomato, concomitantly with increased biosynthesis of lycopene, the processes for splitting into β and γ carotene were absent [16] and the mRNAs of *CrtL-b* and *CrtL-e*, which encode lycopene β-cyclase and ε-cyclase, enzymes involved in the cyclization of lycopene that leads to the formation of β respectively γ carotene, are strongly down-regulated during fruit ripening [28] The absence of cyclization and splitting contributes to the accumulation of lycopene in tomato fruit. Starch transiently accumulates in young tomato fruit and undergoes almost complete degradation by

maturity. In fact starch accumulation results from an unbalance between synthesis and degradation. Enzymes capable of degrading starch have been detected in the plastids of tomato fruit. In addition, tomato fruit can synthesize starch during the period of net starch breakdown, illustrating that these two mechanisms can coexist [30]. Starch synthesis enzymes have been encountered in the tomato chromoplast, but many of the proteins of starch breakdown were also detected suggesting a prevalence of the degradation process [16] In terms of reactive oxygen species, antioxidant enzymes are up-regulated during chromoplast development and lipids, rather than proteins, seem to be a target for oxidation. In the chromoplasts an up-regulation in the activity of superoxide dismutase and of components of the ascorbate-glutathione cycle was observed [31].

The plastid-to-nucleus signaling also undergoes important changes. In the chromoplast the main proteins involved in the synthesis of Mg-protoporphyrin IX, a molecule supposed to play an important role in retrograde signaling [32] are absent, but other mechanisms such as hexokinase 1 or calcium signaling were be present [16]. The plastid-nucleus communication is still an open subject with many still unanswered questions.

2. *A number of metabolic pathways are conserved during chromoplast differentiation*

The comparison of data arising from proteomics of the chloroplast [33] and of the chromoplast [16] as well as biochemical analysis of enzyme activities suggest that several pathways are conserved during the transition from chloroplast to chromoplast. Such is the case for the Calvin Cycle, the oxidative pentose phosphate pathway (OxPPP) and many aspects of lipid and sugar metabolism. Activities of enzymes of the Calvin cycle have been measured in plastids isolated from sweet pepper. They may even be higher in chromoplasts than in chloroplasts [34] In ripening tomato fruits, several enzymes of the Calvin Cycle (hexokinase, fructokinase, phosphoglucoisomerase, pyrophosphate-dependent phosphofructokinase, triose phosphate isomerase, glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate kinase, glucose 6-phosphate dehydrogenase) are active [35]. The activity of glucose 6-phosphate dehydrogenase (G6PDH), a key component of the OxPPP was higher in fully ripe tomato fruit chromoplasts than in leaves or green fruits [36]. Also, a functional oxidative OxPPP has been encountered in isolated buttercup chromoplasts [37]. Proteomic analysis have demonstrated that an almost complete set of proteins involved in the OxPPP are present in isolated tomato fruit chromoplasts (Figure 2). The persistence of the Calvin cycle and the OxPPP cannot be related to photosynthesis since the photosynthetic system is disrupted. In non-photosynthetic plastids the Calvin cycle could provide reductants and also precursors of nucleotides and aromatic aminoacids to allow the OxPPP cycle to function optimally [16].



1	SGN-U312791	phosphoribulokinase
2	SGN-U315528	ribose 5-phosphate isomerase
3	SGN-U313308	ribulose-phosphate 3-epimerase
4	SGN-U312320	transketolase
4	SGN-U312319	transketolase
4	SGN-U312322	transketolase
4	SGN-U323721	transketolase
5	SGN-U315559	sedoheptulose-bisphosphatase
6	SGN-U312320	transketolase
6	SGN-U312319	transketolase
6	SGN-U312322	transketolase
6	SGN-U323721	transketolase
7	SGN-U316424	fructose-1,6-bisphosphatase
8	SGN-U314788	fructose-bisphosphate aldolase
8	SGN-U314787	fructose-bisphosphate aldolase
8	SGN-U312608	fructose-bisphosphate aldolase
8	SGN-U312609	fructose-bisphosphate aldolase
8	SGN-U312344	fructose-bisphosphate aldolase
9	SGN-U313729	triose-phosphate isomerase
10	SGN-U312802	glyceraldehyde-3-phosphate dehydrogenase B su
10	SGN-U312804	glyceraldehyde-3-phosphate dehydrogenase B su
10	SGN-U312461	glyceraldehyde-3-phosphate dehydrogenase A su
11	SGN-U313176	phosphoglycerate kinase
12	SGN-U316742	chaperonin 60 beta
12	SGN-U312543	Rubisco activase
12	SGN-U312544	Rubisco activase
12	SGN-U312538	60 kDa chaperonin alpha subunit
12	SGN-U312542	60 kDa chaperonin alpha subunit
13	SGN-U314262	ribulose bisphosphate carboxylase small chain 1A
13	SGN-U314254	ribulose bisphosphate carboxylase small chain 1A
13	SGN-U314701	ribulose bisphosphate carboxylase small chain 3B
13	SGN-U314722	ribulose bisphosphate carboxylase small chain 3B
13	SGN-U314700	ribulose bisphosphate carboxylase small chain 3B
13	SGN-U338973	ribulose bisphosphate carboxylase small chain 3B
14	SGN-U344009	large subunit of RUBISCO
14	SGN-U346314	large subunit of RUBISCO

Figure 2: Presence of proteins of the Calvin cycle in the tomato chromoplastic proteome. Proteins are indicated by black squares and represented by their generic name and unigene SGN code. Numbers represent the position of the protein in the cycle. Data are derived from Barsan et al., [16].

In chloroplasts, thylakoid membranes, as well as envelope membranes, are rich in galactolipids and sulfolipids [38]. Lipid metabolism is also highly active in the chromoplasts. Despite thylakoid disassembly new membranes are synthesized such as those participating in the formation of carotenoid storage structures. These newly synthesized membranes do not derive from the thylakoids but rather from vesicles generated from the inner membrane of the plastid [39]. Key proteins for the synthesis of phospholipids, glycolipids and sterols were identified [16] along with some proteins involved in the lipoxygenase (LOX) pathway. They have been described in the chloroplast and they lead to the formation oxylipins, which are important compounds for plant defense responses [40]. In the tomato chromoplast all proteins potentially involved in the LOX pathway leading to the generation of aroma volatiles were found [16].

The shikimate pathway corresponding to the biosynthetic route to the aromatic amino acids phenylalanine, tyrosine, and tryptophan [41] is active in the chromoplasts of wild buttercup [37] and a number of proteins involved in this pathway have been encountered in the tomato chromoplast [16] and in the bell pepper chromoplast [14].

During fruit ripening an increased synthesis of α-tocopherol was observed [42]. The biosynthesis of α-tocopherol was localizated in the envelope membranes of the *Capsicum annum*

[43] and the almost complete set of proteins of the pathway were present in the tomato chromoplast [16]. The accumulation of α–tocopherol, by protecting membrane lipids against oxidation may contribute to delaying senescence [44].

3. Plastoglobuli, plastoglubulins and the chloroplast to chromoplast transition

Plastoglobules are lipoprotein particles present in chloroplasts and other plastids. They have been recently recognized as participating in some metabolic pathways [45]. For instance, platoglobules accumulate tocopherols and harbor a tocopherol cyclase, an enzyme catalyzing the conversion of 2,3-dimethyl-5-phytil-1,4-hidroquinol to γ-tocopherol [46]. Plastoglobuli also accumulate carotenoids as crystals or as long tubules named fibrils [47,48]. Part of the enzymes involved in the carotenoid biosyntesis pathway (ζ-carotene desaturase, lycopene β cyclase, and two β-carotene β hydroxylases) were found in the plastoglobuli [49].

Plastoglobules arise from a blistering of the stroma-side leaflet of the thylakoid membrane [50] and they are physically attached to it [45]. In the chromoplast a change in the size and number of plastoglobuli was observed. They are larger and more numerous than in the chloroplast [7]. Plastoglobulins are the predominant proteins of plastoglobules. Several types of plastoglobulins have been described: fibrillin, plastid-lipid associated proteins (PAP) and carotenoid-associated protein (CHRC). All plastoglobulins participate in the accumulation of carotenoids in the plastoglobule structure. Carotenoids accumulate as fibrils to form supramolecular lipoprotein structures. A model for fibril assembly has been proposed in which the core is occupied by carotenoids that interact with polar galacto- and phospho-lipids. Fibrillin molecules are located at the periphery in contact with the plastid stroma [51]. In tomato the over-expression of a pepper fibrillin caused an increase in carotenoid and carotenoid-derived flavour volatiles [39] along with a delayed loss of thylakoids during the chloroplast to chromoplast transition. In fibrillin over-expressing tomato the plastids displayed a typical chromoplastic zone contiguous with a preserved chloroplastic zone. PAP, is another major protein of plastoglobules that also participates in the sequestration of carotenoids [51,52]. As for CHRC, its down-regulation resulted in a 30% reduction of carotenoids in tomato flowers [53]. Plastoglobuli are therefore complex assemblies that play key a role in carotenoid metabolism and greatly influence the evolution of the internal structure of the plastid during the chloroplast to chromoplast transition

4. A key player in chromoplast differentiation: the Or gene

The *Or* gene was discovered in cauliflower where the dominant mutation *Or* conferred an orange pigmentation with accumulation of β-carotene mostly in the inflorescence. [54]. The *Or* gene

was isolated by positional cloning [55]. It is localized in the nuclear genome and is highly conserved among divergent plant species [56]. The Or protein corresponds to plastid-targeted a DnaJ-like co-chaperone with a cysteine-rich domain lacking the J-domain [55]. DnaJ proteins are known for interacting with Hsp70 chaperones to perform protein folding, assembly, disassembly and translocation. The *Or* mutation is not a loss of function mutation as indicated by the absence of phenotype upon RNAi silencing. It is probably a dominant-negative mutation affecting the interaction with Hsp70 chaperones [57].The *OR* mutants displayed an arrest in plastid division so that a limited number of chromoplasts (one or two) were present in the affected cells [58]. Potato tubers over-expressing the *Or* gene accumulate carotenoids [56]. In the *OR* mutant the expression of carotenoid biosynthetic genes was unaffected and chromoplasts differentiated normally with membranous inclusions of carotenoids similar to those of carrot roots. It is concluded that the *Or* gene is not involved in carotenoid biosynthesis but rather creates a metabolic sink for carotenoid accumulation through inducing the formation of chromoplasts [59].

5. ***Transcriptional and translational activity in the plastid undergo subtle changes during chromoplast biogenesis***

Most proteins present in the plastid are encoded by nuclear genes. The plastid genome encodes around 84 proteins [60]. Restriction enzyme analysis between chloroplasts of leaves and chromoplasts of tomato fruit indicate the absence of re-arrangements, losses or gains in the chromoplastic DNA [61]. During chromoplast differentiation the global transcriptional activity is stable, except for a limited number of genes such as *accD*, encoding a subunit of the acetyl-CoA carboxylase involved in fatty acid biosynthesis, *trnA* (tRNA-ALA) and *rpoC2* (RNA polymerase subunit) [15]. Polysome formation within the plastids declined during ripening suggesting that, while the overall RNA levels remain largely constant, plastid translation is gradually down-regulated during chloroplast-to-chromoplast differentiation. This trend was particularly pronounced for the photosynthesis gene group. A single exception was observed, the translation of *accD* stayed high and even increased at the onset of ripening [15].

Specific studies of few plastid localized genes have been carried out. Genes involved in photosynthesis were, as expected, down-regulated during chromoplast formation [25]. However an up-regulation of the large subunit of *ribulose-1,5-bisphosphate carboxylase/oxygenase* and the 32 kD *photosystem II quinone binding protein* genes has been observed in the chromoplasts of squash fruits (*Cucurbitae pepo*) [62]. A possible explanation would be that these genes could be regulated independently from the plastid differentiation processes. Genes involved in carotenoid biosynthesis

such as the *lycopene β-cyclase* (*CYCB*) were up-regulated during chromoplast formation in many plants including the wild species of tomato *Solanum habrochaites* [63].

6. Changes in gene expression during chromoplast differentiation in ripening tomato

The availability of proteomic data of tomato chromoplats [16] and expression data of a wide range of tomato genes (The Tomato Expression Database: http://ted.bti.cornell.edu [64] allowed classifying genes encoding chromoplastic proteins according to their expression pattern (Table 1). Among the 87 unigenes whose encoded proteins are located in the chromoplast, the biggest functional class corresponds to genes involved in photosynthesis. Most of them (18 out of 24) are either permanently (Table 1C) or transiently (Table 1E) down-regulated at the breaker stage. This is in agreement with the dramatic decrease in the photosynthetic activity of the chromoplast. Only few of the genes involved in photosynthesis had different patterns of expression, i.e up-regulated (Table 1A: U313693 ATP synthase delta chain; U312985 glycine cleavage system H protein; Carboxylase/-oxygenase activase; U312532 oxygen-evolving enhancer protein) or unchanged (Table 1B: U312690 plastocyanin; U312593 chlorophyll A-B binding protein 4; U314994 phosphoglycolate phosphatase). In the case of Calvin Cycle, 5 out of 12 genes (U312344 fructose-bisphosphate aldolase; U312608 fructose-bisphosphate aldolase; U312609 fructose-bisphosphate aldolase; U314254 ribulose bisphosphate carboxylase small chain 1A; U314701 ribulose bisphosphate carboxylase small chain 3B) had a constant decrease during chromoplast differentiation (Table 1C). In tomato fruit the activity of the ribulose-1,5-bisphosphate carboxylase/oxygenase had a constant decrease during fruit ripening [65], which is in line with the transcriptomic and proteomic data. The genes encoding fructose-bisphosphate aldolase isoforms presented different expression profiles being either up- (U314788) or down- (U312344) regulated during tomato fruit ripening. An increase in overall transcript levels for the fructose-1,6-bisphosphate aldolase has been described during ripening [66]. The importance of transcripts and enzyme activity of the various isoforms are unknown. The remaining genes involved in the Calvin cycle showed either increased (Table 1B; U312802 glyceraldehyde-3-phosphate dehydrogenaseB; U312538 RuBisCO subunit binding-protein) or unchanged expression (Table 1A; U316424 fructose-1,6-bisphosphatase; U312544 ribulose bisphosphate). Three genes coding for the OxPPP were found: two of them exhibited a transient increase in expression at the breaker stage (Table 1D: U315528 ribose 5-phosphate isomerase-related; U332994 6-phosphogluconate dehydrogenase family protein) and one a transient decrease (Table 1E: U315064 transaldolase). The 3 genes involved in tetrapyrolle biosynthesis are not part of the chlorophyll synthesis branch and all of them had an increased expression (Table 1B: U315993 coproporphy -rinogen III oxidase; U315267 uroporphyrinogen decarboxylase; U315567

hydroxymethylbilane synthase), suggesting that the synthesis of tetrapyroles continues during the transition from chloroplast to chromoplast. As expected, most of the genes (5 out of 6) coding for enzymes involved in carotenoid synthesis showed continuous (Table 1B: U314429 phytoene synthase; U315069 isopentenyl-diphosphate delta-isomerase II; U316915 geranylgeranyl pyrophosphate synthase; U318137 phytoene dehydrogenase) or transient (Table 1 D: U313450 geranylgeranyl reductase) up-regulation. The precursors for carotenoid production are synthesized through the methylerythritol phosphate (MEP) pathway. The gene encoding hydroxymethylbutenyl 4-diphosphate synthase (HDS) (U314139) downstream in the pathway has stable expression (Table 1A). This is consistent with previous studies that showed that there were no significant changes in *HDS* gene expression during tomato fruit ripening [67].

In the case of lipid metabolism three genes showed increased expression (Table 1B: U315474 3-oxoacyl-(acyl-carrier-protein) synthase I; U315475 3-oxoacyl-(acyl-carrier-protein) synthase I; U313753 pyruvate dehydrogenase E1 component) and two genes had transient increase (Table 1D: U315697 enoyl-(acyl-carrier protein) reductase (NADH) U321151 lipoxygenase). Phosphatidylglycerol phosphate synthase showed decreased expression (Table 1C). This enzyme is involved in the biosynthesis of phosphatidylglycerol and is considered as playing an important role in the ordered assembly and structural maintenance of the photosynthetic apparatus in thylakoid membranes and in the functioning of the photosystem II [68]. The down-regulation of this gene during chromoplast differentiation is consistent with thylakoid disintegration and photosynthesis disappearance.

Four genes of the starch metabolism present up-regulation (Table 1B). Two of them are part of the starch biosynthesis (U312423 1,4-alpha-glucan branching enzyme; U312427 1,4-alpha-glucan branching enzyme) and one of them is involved starch degradation (U315116 starch excess protein (SEX1). The fourth one, an isoamylase (U333011) can participate of both starch degradation or synthesis, depending on the isoform. The expression of the gene that codes a starch degrading glucan phosphorylase (U316416) decreases and the expression of another starch degrading gene, beta-amylase (U313315), has a negative transient expression. In addition proteomic studies have shown the presence of two starch excess proteins (SEX1 and 4) that probably contribute to the absence of starch accumulation [16]. Starch is degraded during the chloroplast to chromoplast transition to provide carbon and energy necessary to sustain the metabolic activity during fruit ripening. Several enzymes are responsible for the processes, each one possessing several isoforms with different regulatory mechanisms [69].

Interestingly genes involved in aroma production such as *ADH* (U314358) or *LOXC* (U315633) had a constant increase in gene expression (profile B). This could be related to the increase in aroma production via the LOX pathway.

The microarray data discussed in this section cover a wide range of the tomato transcriptome. However, several isoforms of several genes are not represented in the database, which could explain some contradictory patterns of expression encountered in our analysis. Nevertheless, although not providing a full picture of the molecular events occuring during the chloroplast-to-chromoplast transition, these data confirm the regulation at the transcriptional level of the most salient events.

Table 1: Expression profile analysis of 87 genes whose products are targeted to tomato chromoplasts (*)

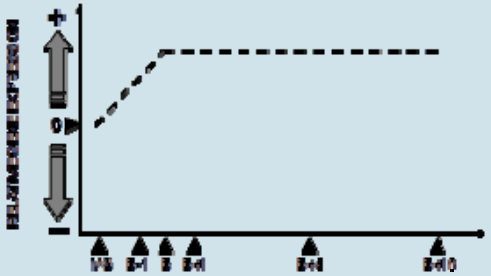
A 	**Photosystem:** U313693 ATP synthase delta chain; U312985 glycine cleavage system H protein; Carboxylase/-oxygenase activase; U312532 oxygen-evolving enhancer protein; **Calvin cycle:** U316424 fructose-1,6-bisphosphatase; U312544 ribulose bisphosphate **Secondary metabolism:** U314139 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase.
B 	**Photosystem:** U312690 plastocyanin; U312593 chlorophyll A-B binding protein 4; U314994 phosphoglycolate phosphatase, **Calvin cycle:** U314788 fructose-bisphosphate aldolase; U312802 glyceraldehyde-3-phosphate dehydrogenaseB; U312538 RuBisCO subunit binding-protein; **Redox:** U314092 L-ascorbate peroxidase; U319145 thioredoxin family protein; U320487 monodehydroascorbate reductase **Amino acid metabolism:** U321505 anthranilate synthase; U317466 3-phosphoshikimate 1-Carboxyvinyltransferase; U317564 tryptophan synthase, **Lipid metabolism:** U315474 3-oxoacyl-(acyl-carrier-protein) synthase I; U315475 3-oxoacyl-(acyl-carrier-protein) synthase I; U313753 pyruvate dehydrogenase E1 component, **Major CHO metabolism:** U315116 starch excess protein (SEX1); U333011 isoamylase, putative; U312423 1,4-alpha-glucan branching enzyme; U312427 1,4-alpha-glucan branching enzyme, **Secondary metabolism:** U314429 phytoene synthase; U315069 isopentenyl-diphosphate delta-isomerase II; U316915 geranylgeranyl pyrophosphate synthase; U318137 phytoene dehydrogenase **Tetrapyrrole synthesis:** U315993 coproporphy -rinogen III oxidase; U315267 uroporphyrinogen decarboxylase; U315567 hydroxymethylbilane synthase, **Mitochondrial electron transport:** U316255 NADH-ubiquinone oxidoreductase, **Fermentation, ADH:** U314358 alcohol dehydrogenase (ADH) **Miscellaneous, cytochrome P450:** U313813 NADPH-cytochrome p450 reductase, **S-assimilation. APS:** U313496 sulfate adenylyltransferase 1 **Development unspecified:** U316277 senescence-associated protein (SEN1) **Cell organisation:** U313480 plastid-lipid associated protein PAP, putative **Hormone metabolism:** U315633 lipoxygenase **N-metabolism ammonia metabolism:** U323261 glutamate synthase (GLU1) **Stress abiotic heat:** U315717 HS protein 70. **Not assigned, No ontology:** U317890 hydrolase, alpha/beta fold family protein

Table 1: Cont.

C 	**Photosystem:** U312531 oxygen-evolving enhancer protein; U313447 photosystem I reaction center subunit IV; U313204 chlorophyll A-B binding protein 2; U313245 ATP synthase gamma chain 1; U312436 chlorophyll A-B binding protein; U313211 chlorophyll A-B binding protein 2; U313212 chlorophyll A-B binding protein 2; U313213 chlorophyll A-B binding protein 2; U312572 photosystem II oxygen-evolving complex 23 (OEC23); U314260 photosystem I reaction center subunit III family protein **Calvin cycle:** U312344 fructose-bisphosphate aldolase; U312608 fructose-bisphosphate aldolase; U312609 fructose-bisphosphate aldolase; U314254 ribulose bisphosphate carboxylase small chain 1A; U314701 ribulose bisphosphate carboxylase small chain 3B; **Lipid metabolism:** U319207 phosphatidylglycerol phosphate synthase (PGS1) **Redox:** U313537 dehydroascorbate reductase **Major CHO metabolism:** U316416 glucan phosphorylase, putative **N-metabolism:** U314517 glutamine synthetase (GS2) **Amino acid metabolism:** U317344 bifunctional aspartate kinase/homoserine dehydrogenase; U320667 cystathionine beta-lyase
D 	**Redox:** U314061 peroxiredoxin Q; U314093 L-ascorbate peroxidase, thylakoid-bound (tAPX); U314923 2-cys peroxiredoxin, **OPP, Non-reductive PP:** U315528 ribose 5-phosphate isomerase-related; U332994 6-phosphogluconate dehydrogenase family protein; U316131 6-phosphogluconate dehydrogenase NAD-binding domain-containing protein **Secondary metabolism:** U317741 acetyl co-enzyme A carboxylase carboxyl transferase alpha subunit family; U313450 geranylgeranyl reductase **Amino acid metabolism:** U317245 tryptophan synthase-related; **N-metabolism ammonia metabolism:** U317524 ferredoxin-nitrite reductase; **Lipid metabolism:** U315697 enoyl-(acyl-carrier protein) reductase (NADH) U321151 lipoxygenase. **Transport metabolite:** U312460 triose phosphate/phosphate translocator, putative **Signalling calcium:** U315961 calnexin 1 (CNX1)
E 	**Photosystem:** U312843 chlorophyll A-B binding protein; U312858 cytochrome B6-F complex iron-sulfur subunit; U313214 chlorophyll A-B binding protein 2; U312791 phosphoribulokinase (PRK); U317040 photosystem II reaction center PsbP family protein; U312449 chlorophyll A-B binding protein CP26; U312661 chlorophyll A-B binding protein CP29 (LHCB4); U313789 ATP synthase family **Calvin cycle:** U312461 glyceraldehyde 3-phosphate dehydrogenase A; U312871 oxygen-evolving enhancer protein 3; **Redox:** U315728 glutathione peroxidase, **OPP non-reductive PP transaldolase:** U315064 transaldolase, **Signaling calcium:** U318939 calcium-binding EF hand family protein, **Major CHO metabolism:** U313315 beta-amylase.

(*)Genes represented in this table are filtered from TED database crossing with the proteins described by Barsan et al 2010. The expresssion profiles were clustered with of the Mathlab –MathWorks software package and further reduced to five expression profiles according to their general tendencies represented in the first column. The expression values used in this analysis were taken from experiment E011 from TED database. Relative expression refers to ratio between the expression values of each ripening point and MG. All data were normalised by the mean and log2 transformed. **(A)** genes that remain stable during ripening, **(B)** genes that have an increase or **(C)** a decrease until breaker stage and then reach a plateau, **(D)** genes that have a positive or **(E)** negative transient expression around the breaker stage. (MG, mature green; B-1, one day before breaker; B, breaker stage; B+1, one day after breaker; B+5, five days after breaker; B+10, ten days after breaker)

7. Conclusions and perspectives

With the advent of high throughput technologies great progress has been made in the recent years in the elucidation of the structure and function of plastids. The most important data obtained in the area have been generated for the chloroplast of Arabidopsis. Much less information is available for the chromoplast. However recent studies with bell pepper [14] and tomato fruit [16] have allowed assigning to chromoplasts a number of proteins of around 1 000, which is in the same order of magnitude as Arabidopsis chloroplasts [33]. This number is however much lower than the number of proteins predicted to be located in the plastid which has been estimated at up to 2700 [70] or even 3800 [71]. The increased sensitivity of the mass spectrometry technologies associated with efficient methods of purification of plastids, particularly chromoplasts, will allow in the future identifying more proteins. So far, changes in the proteome have not been described during the differentiation of chromoplast. Such studies imply the development of efficient protocols for isolating plastids at different stages of differentiation during chromoplastogenesis. The combination of proteomics and transcriptomics may also give novel information on the process in a near future. The discovery of the *Or* gene has been a great step forward to the understanding of the molecular determinism of chromoplast differentiation. There is a need to better understand the regulatory mechanism controlling the expression of the *Or* gene. Many genes encoding for plastidial proteins are regulated by the plant hormone ethylene and therefore participate in the transcriptional regulation of the fruit ripening process in general [72,73]. Other hormones such as ABA and auxin may also be involved. Interactions between hormones and other signals (light, for instance) during chromoplast differentiation represent another field of investigation to be explored. Because most of the proteins present in the chromoplast are encoded by nuclear genes, it will be important in future to better understand the changes occurring in the processes of transport of proteins to the chromoplast. It is suspected that vesicular transport is gaining importance but more experimental evidence is required. Finally the dialog between the nucleus and the chromoplast and the signals involved needs to be explored. So far most of the studies in this area have been carried out with chloroplasts [74].

In conclusion, important steps forward have been made into a better understanding of chromoplast differentiation. Metabolic re-orientations and specific biochemical and molecular events have been clearly identified. It is predictable that more information will arise from the in-depth description of the molecular events occurring during the chloroplast to chromoplast transition using genomic tools.

Acknowledgements Cristina Barsan has received a bursary from the French Embassy in Bucharest (Romania) for a joint “co-supervision” PhD, Isabel Egea a postdoctoral fellowship from “Fundación Séneca” (Murcia, Spain)” and Wanping Bian a bursary from the University of Chongqing (China)

for a PhD. The participation of Eduardo Purgatto was made possible by a sabbatical fellowship postdoctoral from the government of Brazil (CNPq). The authors acknowledge the Midi-Pyrénées Regional Council for financial support.

References

[1] B. Bathgate, M.E. Purton, D. Grierson, and P.W. Goodenough, "Plastid changes during the conversion of chloroplasts to chromoplasts in ripening tomatoes", *Planta,* vol.165, pp. 197-204, 1985.

[2] Y Ben-Shaul, T. Treffry, and S. Klein, "Fine structure studies of carotene body development", *Journal de Microscopie (Paris),* vol. *7*, pp. 265-274, 1968.

[3] M.G. Grilli-Caiola, and A. Canini, "Ultrastructure of chromoplasts and other plastids in *Crocus sativus* L. (Iridaceae)", *Plant Biosystems*, vol. 138, pp. 43-52, 2004.

[4] H.T. Horner, R.A. Healy, G. Ren, D. Fritz, A. Klyne, C. Seames, and R.W. Thronburg, "Amyloplast to chromoplast conversion in developing ornamental tobacco floral nectaries provides sugar for nectar and antioxidants for protection", *American Journal of Botany,* vol. 94, pp.12-24, 2007.

[5] W.W.Thomson, and J.M. Whatley, "Development of non-green plastids", *Annual Review of Plant Physiology*, vol. 31, pp. 375-394, 1980.

[6] B. Camara, P. Hugueney, F. Bouvier, M. Kuntz, and R. Monéger, "Biochemistry and molecular biology of chromoplast development", *International Review of Cytology*, vol 163, pp.175–247, 1995.

[7] W.M. Harris, and A.R. Spurr, "Chromoplasts of tomato fruit. II. The red tomato", **American Journal** *of* **Botany, *vol.*** 56, pp. 380-389, 1969.

[8] J.A Laborde, and A.R. Spurr, "Chromoplast ultrastructure as affected by genes controlling grana retention and carotenoids in fruits of *Capsicum annuum"*, *American Journal of Botany*, vol. 60, pp. 736-744, 1973.

[9] N. Ljubesic, M. Wrischer, and Z. Devise, "Chromoplasts-the last stages in plastid development", *International Journal of Developmental Biology,* vol.35, pp.251-258, 1991.

[10] M.R. Marano, E.C. Serra, G. Orellano, and N. Carrillo, "The path of chromoplast development in fruits and flowers", *Plant Science,* vol. 94, pp. 1-17, 1993.

[11] F. Bouvier, and B. Camara, "The role of plastids in ripening fruits", In *The structure and functions of plastids*, R.R.Wise, and J.K. Hoober, Eds., pp. 419-432. Springer, Dordrecht. 2007.

[12] K. Pyke, “Plastid biogenesis and differentiation”, in Cell and Molecular Biology of Plastids, R. Bock, Ed., *Topics in Current Genetic*, vol. 19, pp.1-28, Springer-Verlag, Berlin, 2007.

[13] E. Lopez-Juez, “Plastid biogenesis, between shadow and light”, *Journal Experimental Biology*, vol. 58, pp. 11-26.

[14] M.A. Siddique, J. Grossmann, W. Gruissem, and S. Baginsky, “Proteome analysis of bell pepper (*Capsicum annuum* L.) chromoplasts. *Plant and Cell Physiology*, vol. 47, 1663–1673, 2006.

[15] S. Kahlau, and R. Bock, “Plastid transcriptomics and translatomics of tomato fruit development and chloroplast-to-chromoplast differentiation: Chromoplast gene expression largely serves the production of a single protein”, *The Plant Cell* 20: 856-874, 2008.

[16] C. Barsan, P. Sanchez-Bel, C. Rombaldi, I. Egea, M. Rossignol, M. Kuntz, M. Zouine, A. Latché, M. Bouzayen, and J.C. Pech, “Characteristics of the tomato chromoplast proteome revealed by proteomic analysis”. *Journal Experimental of Botany*, vol.61, pp. 2413-2431, 2010.

[17] A.R. Spurr, and W.M.Harris, “Ultrastructure of chloroplasts and chromoplasts in *Capsicum annuum*. I. Thylakoid membrane changes during fruit ripening” *American Journal of Botany*, vol. 55, pp. 1210-1224, 1968.

[18] R.H. Köhler, and M.R. Hanson, “Plastid tubules of higher plants are tissue-specific and developmentally regulated”. *Cell Science*, vol. 113, 81-89, 2000.

[19] M.T. Waters, R.G. Fray, and K.A. Pyke, “Stromule formation is dependent upon plastid size, plastid differentiation status and the density of plastids within the cell”, *The Plant Journal*, vol. 39, pp.655-667, 2004.

[20] P. Jarvis, “Targeting of nucleus-encoded proteins to chloroplasts in plants” *New Phytologist*, vol.179, 257-285. 2008.

[21] S. Miras, D. Salvi, M. Ferro, D. Grunwald, J. Garin, J. Joyard, and N Rolland, “Non-canonical transit peptide for import into the choloroplast”, *Journal of Biological Chemistry*, vol. 227, pp. 4770-4778, 2002.

[22] S. Westphal, J. Soll and U.C. Vothknecht, “A vesicle transport system inside chloroplasts”, *FEBS Letters*, vol. 506, pp. 257–261, 2001.

[23] M.X. Anderson, and A.S. Sandelius, “A chloroplast-localized vesicular transport system: a bioinformatics approach”, *BMC Genomics*, vol.5, pp.40

[24] P.D. Fraser, M.R. Truesdale, C.R. Bird, W. Schuch, P.M. Bramley, “Carotenoid biosynthesis during tomato fruit development”, *Plant Physiology*, 105:405-413, 1994.

[25] A.Y. Cheung, T. McNellis, B. Piekos, “Maintenance of chloroplast components during chromoplast differentiation in the tomato mutant Green Flesh”, *Plant Physiology*, vol. 101, pp. 1223-1229, 1993.

[26] R. Alba, Z. Fei, P. Payton, Y. Liu, S.L. Moore, *P.* Debbie, J.S. Gordon, J.K.C. Rose, G. Martin, S.D. Tanksley, M. Bouzayen, M.M. Jahn, and J.J. Giovannoni, “ESTs, cDNA microarrays, and gene expression profiling: tools for dissecting plant physiology and development”, *The Plant Journal*, vol. 39, pp.697–714, 2004.

[27] S. Carrara, A. Pardossi, G.F. Soldatini, F. Tognoni, and L. Guidi, “Photosynthetic activity of ripening tomato fruit”, *Photosynthetica*, vol. 39, pp.75-78, 2001.

[28] G. Ronen, M. Cohen, D. Zamir, and J. Hirschberg, “Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon cyclase is down regulated during ripening and is elevated in the mutant *delta*”, *The Plant Journal*, vol. 17, pp.341-3511, 1999.

[29] Y. Libal-Weksler, M. Vishnevetsky, M. Ovadis, H. Itzhaki, H. and A.Vainstein, “Flower-specific carotenoid accumulation in chromoplasts: molecular control of carotenoid-associated proteins” *Acta Horticulturae* vol.420, pp.32-34, 1995.

[30] K.Luengwilai, and D.M. Beckles, “Starch granules in tomato fruit show a complex pattern of degradation”, *Journal of Agricultural and Food Chemistry*, vol. 57, pp. 8480–8487, 2009.

[31] M.C. Marti, D. Camejo, E. Olmos, L.M. Sandalio, N. Fernandez-Garcia, A. Jimenez, and F. Sevilla, “Characterisation and changes in the antioxidant system of chloroplasts and chromoplasts isolated from green and mature pepper fruits. *Plant Biosystems*, vol.11, pp. 613-624, 2009.

[32] A.Strand, T. Asami, J. Alonso J.R. Ecker, and J. Chory, “Chloroplast to nucleus communication triggered by accumulation of Mg-protoporphyrinIX”, *Nature*, vol. 421, 79-83, 2003.

[33] M. Ferro, S. Brugière, D. Salvi, D. Seigneurin-Berny, M. Court, L. Moyet, C. Ramus, S. Miras, M. Mellal, S. Le Gall, S. Kieffer-Jaquinod, C. Bruley, J. Garin, J Joyard, C. Masselon, and N. Rolland, N. “AT_CHLORO, a comprehensive chloroplast proteome database with subplastidial localization and curated information on envelope proteins”, *Molecular and Cellular Proteomics*, vol. 9, pp. 1063-1084, 2010.

[34] E.Thom, T. Mohlmann, P.W. Quick, B. Camara, and H.E. Neuhaus, “Sweet pepper plastids: enzymic equipment, characterisation of the plastidic pentose-phosphate pathway, and transport of phosphorylated intermediates across the envelope membrane”, *Planta*, vol. 204, 226–233, 1998.

[35] H. Obiadalla-Ali, A.R. Fernie, J. Kossmann, and J.R. Lloyd, "Developmental analysis of carbohydrate metabolism in tomato (*Lycopersicon esculentum* cv. Micro-Tom) fruits. *Physioogia Plantarum*, vol. 120, pp. 196–204, 2004.

[36] K. Aoki, M. Yamamoto, and K.Wada, "Photosynthetic and heterotrophic ferredoxin isoproteins are colocalized in fruit plastids of tomato", *Plant Physiology*, vol.118, pp. 439-449, 1998.

[37] I.J. Tetlow, C.G. Bowsher, and M.J. Emes, "Biochemical properties and enzymic capacities of chromoplasts isolated from wild buttercup (*Ranunculus acris* L.)", *Plant Science*, vol. 165, pp. 383-394, 2003.

[38] J. Joyard, M. Ferro, C. Masselon, D. Seigneurin-Berny, D Salvi, J Garin, and N. Rolland, Chloroplast proteomics highlights the subcellular compartmentation of lipid metabolism. *Progress in Lipid Research*, vol.49, pp.128-158, 2010.

[39] A.J. Simkin, J. Gaffé, J.P. Alcaraz, J.P. Carde, P.M. Bramley, P.D. Fraser, and M. Kuntz, "Fibrillin influence on plastid ultrastructure and pigment content in tomato fruit. *Phytochemistry*, vol. 68, pp. 545-1556, 2007.

[40] T. Farmaki, M. Sanmartın, P. Jimenez, M. Paneque, C. Sanz, G. Vancanneyt, J. Leon, and J.J. Sanchez-Serrano, "Differential distribution of the lipoxygenase pathway enzymes within potato chloroplasts", *Journal of Experimental Botany*, vol. 58, pp. 555–568, 2007.

[41] K.M. Herrmann, "The shikimate pathway: early steps in the biosynthesis of aromatic compounds", *The Plant Cell*, vol. 7, pp. 907-919, 1995.

[42] H.K. Lichtenthaler, "Zur Synthese der lipophilen Plastidenchinone und Sekundarcarotinoide wahrend der Chromoplastenentwicklung. *Berichte der Deutschen Botanischen Gesellschaft*, vol.82, 483–97, 1969.

[43] Y. Arango, and K.P. Heise, "Localization of a-tocopherol synthesis in chromoplast envelope membranes of Capsicum annuum L. fruits", *Journal Experimental of Botany*, vol. 49, pp. 1259–1262, 1998.

[44] B. Camara, F. Bardat, O. Dogbo, J. Brangeon, and R Moneger, "Terpenoid metabolism in plastids. Isolation and biochemical characteristics of *Capsicum annuum* chromoplasts", *Plant Physiology*, vol. 73, pp. 94–99, 1983.

[45] C Bréhélin, and F. Kessler, "The plastoglobule: a bag full of lipid biochemistry tricks". *Photochemistry and Photobiology*, vol. 84, pp. 1388-1394, 2008.

[46] F. Kessler, and P.A. Vidi, "Plastoglobule lipid bodies: their functions in chloroplasts and their potential for applications", *Advances in Biochemical* **Engineering** */Biotechnology*, vol. 107, pp.153–172, 2007.

[47] Z. Devide, “Ultrastructural changes of plastids in ripe fruits of *Cucurbita pepo* cv. Olifera”, *Acta Botanica Croatica*, vol. 29, pp. 57-60, 1970.

[48] N. Ljubesic, “The formation of chromoplasts in fruits of *Cucurbita maxima* Duch. *Turbaniformis, Botanical Gazette*, vol. 13, pp.286-290,1977.

[49] A.J. Ytterberg, J.B. Peltier, and K.J. van Wijk, “Protein profiling of plastoglobules in chloroplasts and chromoplasts. A surprising site for differential accumulation of metabolic enzymes”, *Plant Physiology*, vol. 140, pp. 984-997, 2006.

[50] J.R. Austin, E. Frost, P. Vidi, F. Kessler, and L.A. Staehelin, “Plastoglobules are lipoproteins subcompartments of the chloroplast that are permanently coupled to thylakoid membranes and contain biosynthetic enzymes”, *The Plant Cell*, vol. 18, pp.1693-1703, 2006.

[51] J. Deruere, S. Romer, A. d'Harlingue, R.A. Backhaus, M. Kuntz, and B. Camara, “Fibril assembly and carotenoid over-accumulation in chromoplasts: a model for supramolecular lipoprotein structures”, *The Plant Cell*, vol. 6, pp. 119–133, 1994.

[52] J. Pozueta-Romero, F. Rafia, G. Houlne, C. Cheniclet, J.P. Carde, M.L. Schantz, and R. Schantz, R. “A ubiquitous plant housekeeping gene, PAP, encodes a major protein component of bell pepper chromoplasts”, *Plant Physiology*, vol. 115, pp.1185–1194,1997.

[53] Y. Leitner-Dagan, M. Ovadis, E. Shklarman, Y. Elad, D.R. David, and A.Vainstein, “Expression and functional analysis of the plastid lipid-associated protein CHRC suggest its role in chromoplastogenesis and stress”, *Plant Physiology*, vol. 142, pp.233-244, 2006.

[54] L. Li, D.J. Paolillo, M.V. Parthasarathy, E.M. DiMuzio, and D.F.Garvin, A novel gene mutation that confers abnormal patterns of β-carotene accumulation in cauliflower (*Brassica oleracea* var. *botrytis*). *The Plant Journal*, vol. 26, pp.59-67, 2001.

[55] S.Lu, J. van Eck, X. Zhou, A.B. Lopez, D.M. O'Halloran, K.M. Cosman, B.J. Conlin, D.J. Paolillo, D.F. Garvin, J. Vrebalov, L.V. Kochian, H. Küpper, E.D. Earle, J. Cao, and L. Li, “The cauliflower Or Gene encodes a DnaJ cysteine-rich domain containing protein that mediates high levels of ß-carotene accumulation”, *The Plant Cell*, vol.18, pp.34 3594-3605, 2006.

[56] X. Zhou, J. van Eck, and L. Li, “Use of the cauliflower *Or* gene for improving crop nutritional quality”, *Biotechnology Annual Review*, vol. 14, pp. 171-190, 2008.

[57] G. Giuliano, and G. Diretto, “Of chromoplasts and chaperones,” *Trends in Plant Science*, vol. 12, pp. 529-531, 2007.

[58] D.J. Paolillo, D.F. Garvin, and M.V. Parthasarathy, “The chromoplasts of *Or* mutants of cauliflower (*Brassica oleracea* L. var. botrytis)”, *Protoplasma*, vol. 224, pp. 245-253, 2004.

[59] L. Li, and J. van Eck, “Metabolic engineering of carotenoid accumulation by creating a metabolic sink”, *Transgenic Research,* vol. 16, pp. 581-585, 2007.

[60] S. Kahlau, S. Aspinall, J.C. Gray, and R. Bock, “Sequence of the tomato chloroplast DNA and evolutionary comparison of Solanaceous plastid genomes”, *Journal of Molecular Evolution,* vol. 63, pp. 194-207, 2006.

[61] C.M. Hunt, R.C. Hardison, and C.D. Boyer, “Restriction enzyme analysis of tomato chloroplast and chromoplast DNA”, *Plant Physiology*, vol. 82, pp 1145-1147,1986.

[62] S.D. Obukosia, C.M. Richards, and C.D. Boyer, ”Expression of plastid-encoded photosynthetic genes during chloroplast or chromoplast differentiation in *Cucurbitae pepo* L. fruits. *Phytochemistry*, vol. 64, pp. 1213-1221, 2003.

[63] M. Dalal, V. Chinnusamy, and K.C. Bansal, “Isolation and functional characterization of *Lycopene β-cyclase* (*CYC-B*) promoter from *Solanum habrochaites”*, *BioMedCentral Plant Biology,* vol. 10, pp.61-76, 2010.

[64] Z. Fei, X. Tang, R. Alba, and J.J. Giovannoni, “Tomato Expression Database (TED): a suite of data presentation and analysis tools”. *Nucleic Acids Research*, vol. 34, pp. 766–770, 2006.

[65] B.A. Martin, J.A. Gauger, and N.E. Tolbert, “Changes in activity of ribulose-1,5 bisphosphate carboxylase/ oxygenase and three peroxisomal enzymes during tomato fruit development and ripening”, *Plant Physiology*, vol. 63, pp. 486-489, 1979.

[66] B. Piechulla, “Differential expression of nuclear- and organelle-encoded genes during tomato fruit development”, *Planta*, vol.174, pp. 505-512, 1988.

[67] M. Rodriguez-Concepcion, J. Querol, L.M. Lois, S. Imperial, A. Boronat, “Bioinformatic and molecular analysis of hydroxymethylbutenyl diphosphate synthase (GCPE) gene expression during carotenoid accumulation in ripening tomato fruit”, *Planta*, vol. 217, pp. 476–482, 2003.

[68] M. Hagio, Z. Gombos, Z. Várkonyi, K. Masamoto, N. Sato, M. Tsuzuki, and H. Wada, “Direct evidence for requirement of phosphatidylglycerol in photosystem II of photosynthesis*”, Plant Physiology*, vol. 124, pp.795–804, 2000.

[69] A.M. Smith, S.C. Zeeman, S.M. Smith, “Starch degradation”, *Annual Review Plant Biology*, vol. 56, 73-97, 2005.

[70] A.H. Millar, J. Whelan, and I. Small, “Recent surprises inprotein targeting to mitochondria and plastis. Current Opinion in Plant Biology, vol. 9, pp. 610-615.

[71] T. Kleffmann, D. Russenberger, A. von Zychlinski, W. Christopher, K. Sjölander, W. Gruissem, W. Baginsky, “The *Arabidopsis thaliana* chloroplast proteome reveals pathway abundance and novel functions”, *Current Biology*, vol. 14, pp. 354-362.

[72] J.J. Giovannoni, “Molecular regulation of fruit ripening” *Annual Review of Plant Physiology and Plant Molecular Biology.* 52: 725-749, 2001.

[73] J.Pirrello, F. Regad, A. Latché, J.C. Pech, and M. Bouzayen, “Regulation of tomato fruit ripening. In *Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*. *CAB Reviews*, vol. 4, pp. 1-14, 2009.

[74] H.S. Jung and J. Chory, Signaling between chloroplasts and the nucleus: can a systems biology approach bring clarity to a complex and highly regulated pathway? *Plant Physiology*, vol. 152, pp. 453459, 2010.

CHAPTER IV

PROTEIN LOCALISATION AND MONITORING OF CHLOROPLAST TO CHROMOPLAST TRANSITION BY CONFOCAL MICROSCOPY

Introduction

Confocal microscopy is a well know technique that allows observing intact cells and organelles. We used it here in three purposes: 1) determining the location of a set of chosen proteins in the cell 2) characterizing isolated plastid populations and 3) assessing the integrity of the plastidial fraction.

Green fluorescent protein (GFP) is a non-destructive assay that can be easily visualized under UV/blue light without any additional substrate or co-factor. Therefore, it has been widely used to monitor transgene expression and protein localization in a variety of cells and organisms (Majid and Parveez, 2006). It seemed suitable for the localization of 9 proteins out of around 1200 discarded after the curation of the tomato chromoplast proteome and considered with uncertain location. Another point of interest was the characterization and estimation of the survival rate of plastids isolated from tomatoes in different ripening stages: mature green, turning and red. Confocal microscopy has been repeatedly applied for the investigation of chloroplast morphology and structural dynamics in higher plants. It enables capture of sharp images of thin optical sections of living tissues and cells (Skaloud et al., 2004, Skaloud et al., 2005, Uniacke and Zerges, 2009), this is the first time that chromoplasts are described by it.

1. Protein localization in the single cell system by GFP –protein coupling

Introduction

Around 1000 proteins out of 2264 identified by mass spectrometry were confirmed as plastidial. The curation was done on the basis of the presence of plastidial signal peptide (TargetP, Predotar, Ipsort) or the presence of the protein in at least one plastidial database (PPDB, PlProt) or data base dedicated to subcellular localization (SUBA). This method has however several limits. The first limit is that data bases were constructed on the basis of previous experimental evidence in plastid proteomics. The best studied plastid is the chloroplast with more than 1000 described proteins (Zybailov et al., 2008; Ferro et al., 2010). The proteomes of the rice etioplasts (von Zychlinski et al.,

2005), wheat amylopasts (Andon et al., 2002; Balmer et al., 2006), bell pepper chromoplast (Siddique et al., 2006) and tobacco proplastids (Baginsky et al., 2004) were also analyzed but the order of magnitude concerning protein content ranges from 120 to 237. The chromoplast is an unique plastid and even if it shares proteins with the chloroplast it must have unique features that are excluded from these databases, due to lack of experimental evidence. The second limit is that all proteins imported into the plastids do not have a plastid signal. For example the Chloroplast Envelope Quinone Oxidoreductase CeQORH is imported through a non-canonical signal peptide transport (Miras et al., 2002; Nada and Soll, 2004). Another possible transport system could be the intracellular vesicular transport. The transport has been observed in chloroplasts by electron microscop, by the use of effectors of vesicle formation (Westphal et al., 2001) and by bioinformatic means (Andersson and Sandelius, 2004). Stromules represent another import system (Natesan et al., 2005).

In consequence we cannot exclude the hypothesis that among the 1200 discarded proteins there could be important chromoplastic components, undocumented as plastidial at the present. The most suitable method for proving the localization of a given protein is the coupling of the protein with a fluorochrome and its expression in the cell. We have chosen 9 random proteins among the 1200 excluded, and we have investigated their localization.

1.1. Material and methods

1.1.1. The choice of proteins and the primers design

Because a large protein number were not classified in plastidial databases nor had a plastidial transfer signal we decided to test 9 of these proteins with uncertain location (table 1) and we tried to localize them in the tobacco protoplasts after GFP fusion as described by Leclerq et al. (2005).

Table1. List of chromoplastic proteins selected for GFP localization

SGN code	**Description**
SGN-U319047	L-galactose dehydrogenase (L-GalDH)
SGN-U314331	Fructose-bisphosphate aldolase, putative
SGN-U312494	Ras-related GTP-binding family protein
SGN-U316689	Bacterial transferase hexapeptide repeat-containing protein
SGN-U313920	Adenylate kinase
SGN-U313592	Carbonic anhydrase, putative
SGN-U312947	UDP-glucoronosyl/UDP-glucosyl transferase family protein
SGN-U314557	LOX1, lipoxygenase, cytosol
SGN-U314881	Spermidine synthase 2 (SPDSYN2

Two positive controls were used: the chloroplastic protein aspartate amynotransferase (SGN-U315206) (Hatch and Mau, 1973) which followed the same procedures as the 9 tested proteins and Rec A protein that was previously confirmed as plastidial (Cerutti et al., 1992) coupled with the red fluorescence protein (RFP), available in our lab, coexpressed in the protoplasts along with the 9 proteins and the aspartate amynotransferase.

The gene sequences were obtained from the database (http://www.sgn.cornell.edu/). A blast against the *Arabidopsis thaliana* database (http://www.arabidopsis.org/Blast) was done in order to identify the coding regions. The forward and reverse primers were isolated. The forward primers had methionine as starting aminoacid and the reverse primers corresponded to the terminal part of the protein just before the stop codon. (Table 2). To the chosen primers standard sequences were added, thus permitting the assembly of the recombination sites attB corresponding to the recombination sites of the ƛ bacteriophage.

The forward primers: aaaaagcaggcttcXXXXXXXXXXX

The reverse primers: caagaaagctgggtcXXXXXXXXXX

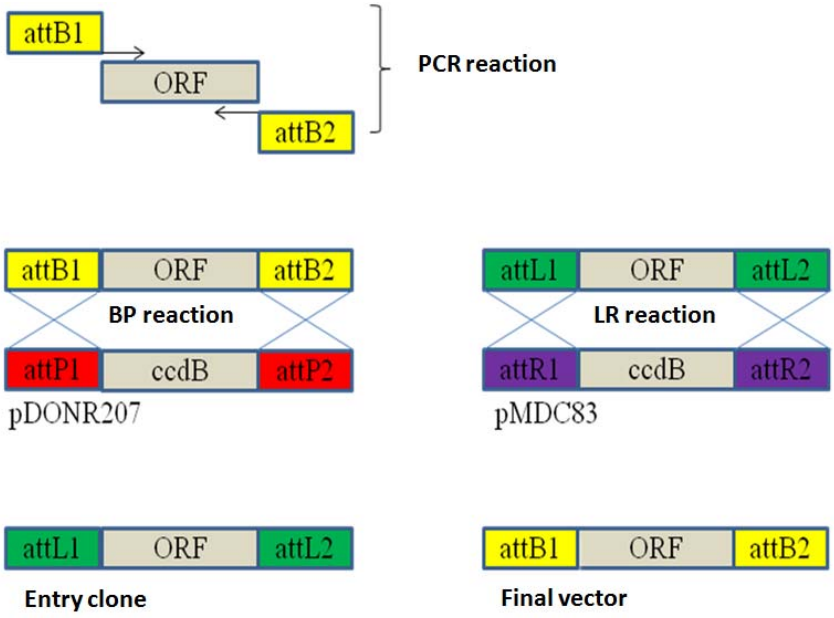
Table 2 List of primers used to amplify the 9 chosen genes

SGN	Primer forward	Primer reverse
SGN-U319047	aaaaagcaggcttcATG GCA GCT CAG ACA TTG CAG CTC CG	caagaaagctgggtcACTTTGTTGGATACCGCTAGGCCATG
SGN-U314331	aaaaagcaggcttcATGTCTTGCTACAAGGGAAAGTATGCTGATG	caagaaagctgggtcATCTTAGTACTTGTAGTCCTTAACATGAAGGCTCTC
SGN-U312494	aaaaagcaggcttcATGGCTGCTCCACCTGCTAGAGCTCG	caagaaagctgggtcAGAACCACAGCAAGCTGATTTTTGGG
SGN-U316689	aaaaagcaggcttcATGGGAACCCTCGGGAAAGCAATTTA	caagaaagctgggtcTTGGGCGATGCTCTTAGCAGCTT
SGN-U313920	aaaaagcaggcttcATG GCT GCT TCA TTA GAA GAT GTT CCT TCA	caagaaagctgggtcTCAAGAAGACAACACATGTTTAACCTCAGAAGT
SGN-U313592	aaaaagcaggcttcATGGCGGAAGATTCATAC GAAGAC GC	caagaaagctgggtcGAGTGCAACAGAAGGAGTAAGTTTGAAGTCAA
SGN-U312947	aaaaagcaggcttcATGGTGCAACCCCATGTCCTATTGGT	caagaaagctgggtcACAACTTTTGCTAACTTGTAGAACAAAAGCTTTTAG
SGN-U314557	aaaaagcaggcttcATGAAGAAAAATGCTCTAGATTTTACTGATCTTGCT	caagaaagctgggtcTATTGACACACTGTTTGGAATTCCTTTGCC
SGN-U314881	aaaaagcaggcttcATG GCA GAT GAG TGT GCT GCT TTT ATG AAG	caagaaagctgggtcTTTTCCTTTGGTTTCGATCACCCTCTTGGC
SGN-U315206	aaaaagcaggcttcATGTTTTCTCTAGCTTCTGTCACTCCTTCAG	caagaaagctgggtcGCTCACATTGTAGTATGAGTCAATGATGGC

1.1.2. Cloning in the Gateway system®

This technology has many advantages such as the transfer in parallel of one or more DNA fragments in various vector types, the fast and efficient cloning of PCR products of various sizes, the conservation of the orientation and of the ORF of the transferred DNA fragment, the obtention of a high percentage (≥ 99%) of transformed colonies, robust reactions taking place in unfriendly conditions and the possibility of automatization of the reaction sequence

The steps of the system can be resumed as showed below:



1.1.3.Gene amplification

For gene amplification an equimoecular cDNA mix coming from fruits of different ripening stages was used. The PCR was performed by using the Phusion® Taq polymerase (New England Biolabs) and the parameters indicated by the producers of the enzyme were strictly followed. The amplified sequences were put on an agarose gel of 1,2% and electrophoresis was performed. The results were visualized after staining with ethidium bromide

1.1.4.The assembly of the recombination sites attB

attB1: 5'-ggggacaagtttgtacaaaaaagcaggcttc-3'

attB2: 5'-gacccagctttcttgtacaaagtggtcccc-3'

The assembly was done by PCR amplification of the products resulted previously.

The entry clone and the transfer of the final construction in the respective vectors was performed with the available Invitrogen kits for BP and LR reactions. We used the pDON207 (fig. 1) vector to create the entry clone and the pMDC83 (fig.2) as final vector.

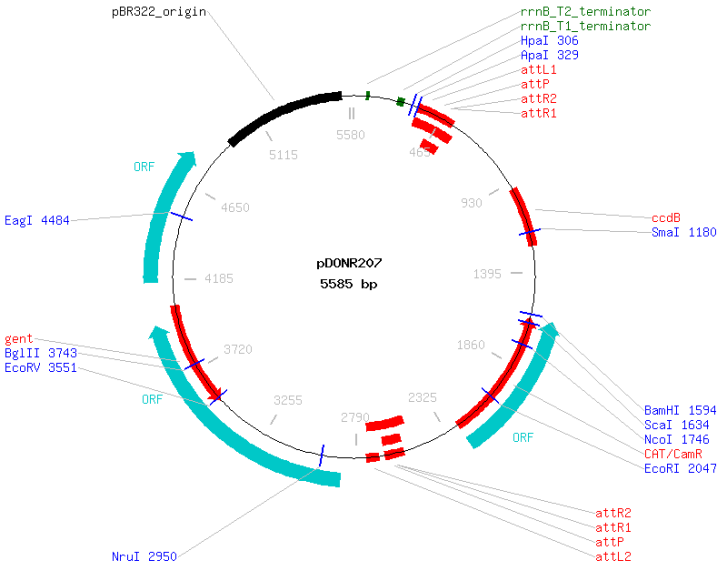


Figure 1: The pDON207 vector

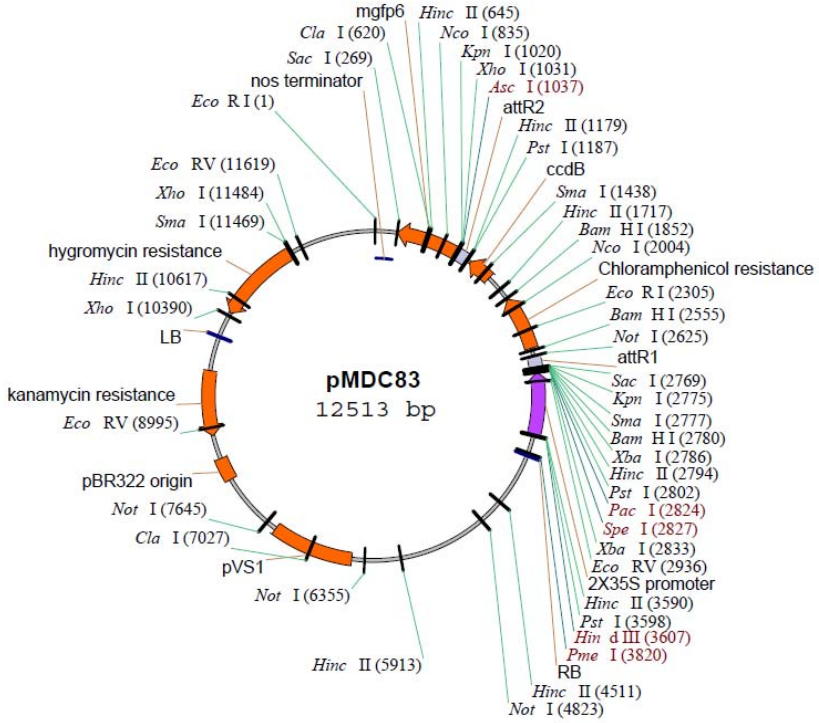


Figure 2: The pMDC83 vector

1.1.5. Protoplast transformation and visualization of the localization of the proteins

We used protoplasts obtained from BY-2 tobacco cells cultivated as described by Abel et al., (1995) with the modifications described by Leclercq et al. (2005). The cells are subcultured once per week for ~ one week old 8 ml of cell suspension in 200 ml of media or ~ 2 ml of cell suspension in 50 ml of media and placed in the culture chamber under gentle agitation, in the dark at 25 ° C.

For 1 litre of culture media:

- MS culture media (Duchefa): 4.3 g
- KH_2PO_4 200 mg
- Sucrose: 30 g
- Myoinositol 100 mg
- Thiamine: 10 mg (1 ml of 10 mg / ml stored in the freezer)
- 2.4 D-180 µg (180 µl of a 1 mg / ml in H_2O stored at 4 ° C)
- pH to 5.8

The culture media was autoclaved in Erlenmeyer flasks plugged with cotton wrapped in gauze. Thiamine and 2,4-D were added after autoclaving and it was stored at -20 ° C.

i) Cell preparation

Twenty ml of cell suspension of tobacco BY-2 of approximately 7 days of culture were placed in a 50 ml Falcon tube and then centrifuged for 5 min at 3500 rpm at room temperature. The supernatant was discarded and the cells were rinsed with - 40 ml of Tris-MES, 25 mM mannitol, 0.6 M, pH 5.5. They were centrifuged for 5 min at 3500 rpm at room temperature, the supernatant was removed by inverting the tube and about 2 g of cells were weight.

ii) Enzyme digestion

Twenty ml of enzyme solution (1% Caylase 345 (CAYLA, 0.2 g / 20 ml); 0.2% pectolyase Y-23 (Serva) (40 mg / 20 ml); 1% BSA (0.2 g / 20 ml); Tris-MES, 25 mM mannitol, 0.6 M, pH 5.5.) were prepared. The solution was stirred about 1 h at room temperature, centrifuged 5 min at 3000 rpm to eliminate and insoluble fraction and then sterilized by filtration with a 0.45 µm filter. Two g of cells were added in a sterile Erlenmeyer flask containing 20 ml of enzyme solution and placed in a shaking water bath (speed 30-40 rpm) at 37 ° C for 1 to 2 hours in the dark. The digestion stage of the cells was checked under a microscope (objective 10X) by placing a drop of cell suspension on a slide after a 90 min digestion.

iii) Purification of protoplasts

The cell suspension was filtered on a sterile nylon cloth of 30 µm directly into a round bottom 50 ml tube and was then diluted with 30 ml of W5 solution (154 mM NaCl; 125 mM $CaCl_2$ $2H_2O$; 5 mM KCl; 5 mM glucose; 0.1% MES, pH 5.6 to 6). It was then centrifuged for 5 min at 1000 rpm (80-100 g) at room temperature. The supernatant was removed by aspiration and the protoplasts were washed in 50 ml of W5. The washing was repeated using only 10 ml of W5 this time.

Protoplasts were counted the on a Fuchs-Rosenthal plate, under the microscope on at least a dozen squares: Protoplast no / ml = protoplast no / squares x 80 000

iv) The transformation

The proptoplast suspension was centrifuged for 3 min at 1000 rpm (80-100 g) at room temperature. The supernatant was discarded and the pellet was resuspended in a volume of mannitol/ Mg buffer (0.55 M mannitol; 15 mM $MgCl_2$, 0.1% MES, pH 5.6) to reach a final concentration of 0.5 to 1106 protoplasts / ml and then incubated on ice for 30 min. All the other steps are performed at room temperature.

Twenty-five µg of carrier DNA (2.5 µl DNA 10mg/ml salmon sperm,Clontech) and 10-25 µg plasmid containing the construction test (purified plasmid Midiprep or MaxiPrep, Promega) were mixed together and then mixed with 150-200 µl protoplast suspension

One volume of PEG solution (40% PEG 4000; 0.1 M $Ca(NO_3)2$ 4 H_2O; 0,5 M mannitol; 0,1 % MES, pH 6,5) was added and the samples were incubated for 30 min at room temperature.Eight hundred µl of W5 solution was added after the incubation and the mixed in by inverting the tube. It was followed by a 10 min at 100 g centrifugation. The supernatant was discarded and the pellet was resuspended in 1,1 ml of W5 solution. The tubes were incubated horizontally at 25 ° C in the dark for 16 hours at under gentle agitation

v) Microscopic observation

The transformed protoplasts in suspension were centrifuged for 5 min at 1000 rpm at room temperature. The supernatanat was discarded and 20 µl pellet were deposed on a lamella. Several drops of mineral oil put on the slide will ensure the watertight integrity of the preparation. The subcellular localization was observed by confocal microscopy, (l = 488 nm) (Leica TCS SP, Leica DM Irbe). .

We used the aspartate aminotransferase and the Rec A protein as internal plastidial markers. The first was marked with GFP and the second with RFP (red fluorescence protein). The success of the transformation was verified by using a pGREEN vector with the GFP protein overexpressed by a 35S promoter.

1.2.Results and discussion

Only one protein (fig. 3) out of nine tested was located in the chloroplasts, two (fructose – bisphosphate aldolase and UDP _ glucoronosyl/UDP – glucosyl transferase) emitted no or weak GFP fluorescence 4 located in the cytoplasm and/or nucleus and Golgi-reticulum, one only in the Golgi-reticulum and one only in the nucleus after a 24h incubation. After a 48h incubation 6 had uncertain location, the adenilate kinase had a plastidial location, two proteins were located in the cytoplasm and/or nucleus and one had only nuclear location (Table 3).

Table.3 The localization of the 9 tested proteins

SGN	Protein	Localisation 24h	Localisation 48h
U319047	L-galactose dehydrogenase (L-GalDH)	Noyau + cytoplasme	Noyau + cytoplasme
U314331	Fructose-bisphosphate aldolase, putative	No fluorescence (codon stop)	nd
U312494	Ras-related GTP-binding family protein	Cytoplasme + Golgi-reticulum + Noyau	nd
U316689	Bacterial transferase hexapeptide repeat-containing protein	weak signal GFP (10 µg DNA)	weak signal GFP
U313920	Adenylate kinase	Chloroplastes	nd
U313592	Carbonic anhydrase, putative	Cytoplasme (no noyau) Golgi /reticulum ?	nd
U312947	UDP-glucoronosyl/UDP-glucosyl transferase family protein	weak signal GFP (10 and 20 µg DNA)	No protos fluo
U314557	LOX1, lipoxygenase, cytosol	Cytoplasme (no noyau, no Golgi ni RE)	Cytoplasme
U314881	Spermidine synthase 2 (SPDSYN2)	Noyau	noyau
U315206	Aspartate aminotransferase, chloroplast, mitochondrion	Chloroplastes and no mitochondrial	Chloroplastes

There are several hypotheses for this outcome. One of them is the experimental model itself, not close enough to the reality of the initial parameters. Tobacco protoplasts from callus cells with chloroplasts lacking chlorophyll were used. The selected proteins were identified in the tomato chromoplast and they have unknown transport system. We can infer the lack of the transport system involved in the transfer of these proteins in the case of the tobacco protoplasts. Useful expression of the GFP cDNA in plants requires that: (i) the GFP apoprotein be produced in suitable amounts within plant cells, and (ii) the nonfluorescent apoprotein undergoes efficient post-translational modification to produce the mature GFP. Another hypothesis is that merging the proteins with the GFP leads to an alteration in its structure, thus leading to an incompatibility with the transport system for the original protein in the cell. Several reports in which the chimeric molecule behaves differently than the native molecule are scant emerged. In the case of the addition of GFP to the C-terminus of granulysin the the signal(s) that cytotoxic lymphocytes use to sort it to the regulated secretory pathway despite its normal biosynthesis and secretion are obscured (Hanson and Ziegler, 2004). The fusion of the GFP tag, which carboxyl terminus of connexin43 (Cx43), altered the gap junction size by masking the carboxyl terminal amino acids of Cx43 (Hunter at al., 2003)

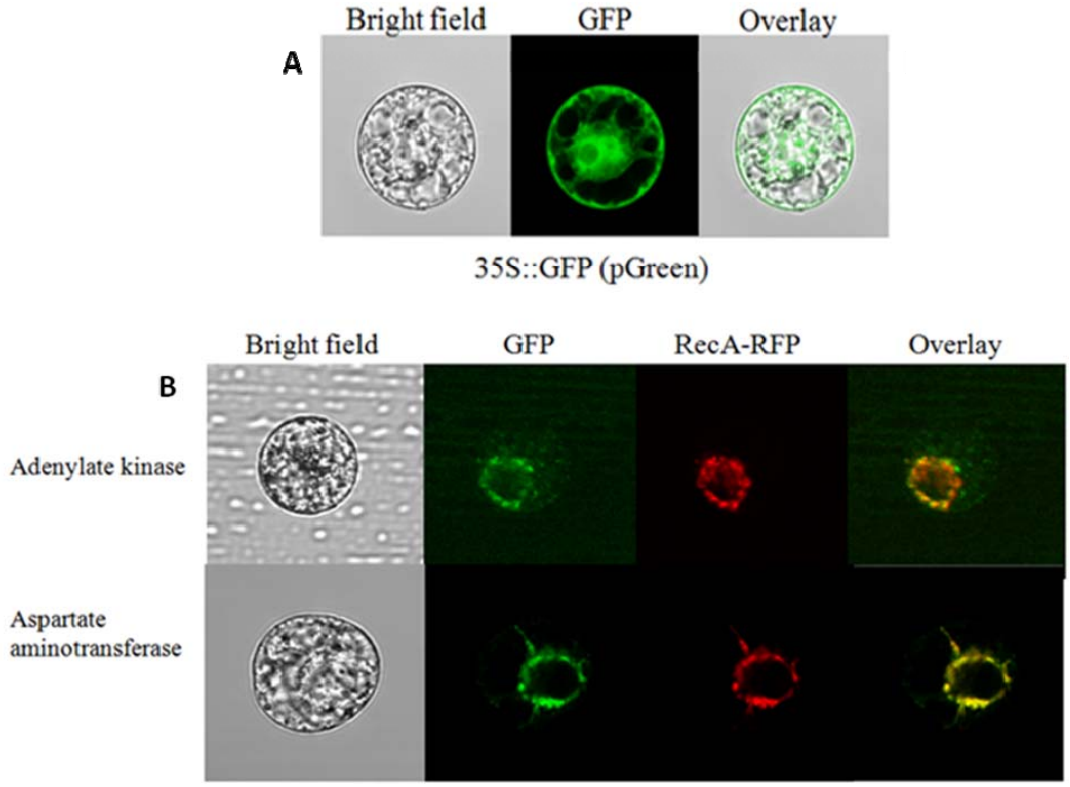


Figure3: Plastidial localization of adelylate kinase in tobacco protoplasts. The expression in the tobacco protoplasts of the: **A)** 35S promoter-driven GFP expression; with a view of the protoplast in the brightfield, the fluorescence corresponding to the GFP and the overlay of the two images **B)** adenilate kinase and the positive markers aspartate aminotransferase and Rec A. The images represent the protoplasts in the bright field, the fluorescene corresponding to GFP and RFP in each protoplast and an overlay of the two types of fluorescence that places both adelitate kinase and the aspartate aminotransferase in the same sub-cellular structures as the RecA protein (the chloroplasts)

In conclusion, the results of the experiments did not shed a light on the localization of the proteins that were not confirmed as plastidial by bioinformatic curation, but considering the limitation of the method we cannot classify them with absolute certainty as contaminants but leave them in a grey area of uncertainty.

2. The chloroplast to chromoplast transition

Introduction

As revealed by the comprehensive survey with the new mass spectrometry technologies, the chromoplast proteome (Barsan et al., 2010) was found to be as complex as the chloroplast's (Zybailov et al., 2008; Ferro et al., 2010). The development of specific protocols to isolate different populations of fruit plastids, the comparison of their proteomes in different stages of fruit ripening is the next step in this field of investigation and can be extremely useful in depicting the details of the plastid differentiation process. So far there are no studies describing the proteome of the tomato chloroplast nor the isolation of plastids in different developmental stages. A challenge in isolating immature chromoplasts is the exploitation of plastid green fluorescent protein (GFP) and confocal microscopy allowing to follow the *in vivo* dynamics of organelles, revealing some aspects of their cell biology. This technology has been primarily used on ripening fruits of tomato, providing significant insights into the morphology of plastid differentiation and chromoplast accumulation (Waters et al., 2004; Forth and Pyke, 2006). The majority of chromoplasts from wild-type tomato fruit probably arise by binary fission of chloroplasts during the green fruit stage up to and including breaker stage prior to differentiation, although budding mechanism has been also observed (Forth and Pyke, 2006). However there are still some doubs concerning the origin of the chromoplast in the tomato fruit so more profound studies on the chloroplast to chromoplast transition process are necessary.

2.1.Material and methods

2.1.1. Fruit sample and plastid isolation

Tomato fruits (*Solanum lycopersicum* cv MicroTom) were germinated and cultivated under greenhouse conditions and collected– as mature green, turning (2 days after breaker) and red (10 days after breaker) stages. Fruits were thoroughly washed with distilled water, the seeds and the gel were eliminated and the pericarp was cut into small pieces (0.5–1.0 cm). Prior to homogenisation, the small fruit pieces were incubated in ice-cold extraction buffer (HEPES 250 mM, sorbitol 330 mM, EDTA 0.5 M, β-mercaptoethanol 5m M pH 7.6) for 30 min. Intact purified plastids were obtained by differential and density gradient centrifugation in discontinuous gradients of sucrose. To isolate chloroplasts and mature chromoplasts three layers sucrose gradients were used (0.9 M-1.15 M-1.45 M and 0.5 M-0.9 M-1.35M of sucrose, respectively). To obtain intact purified immature chromoplasts from turning tomatoes breaker, a more sensitive discontinuous sucrose density gradient was necessary (0.5M-0.9M-1.15M-1.25M-1.35M-1.45M). Intact chloroplasts, immature

chromoplasts and mature chromoplasts were located in the 1.15 M-1.45 M, 0.9 M- 1.15 M and 0.9 M-1.35 M sucrose interfaces, respectively. The collected plastidial bands were washed twice with extraction buffer (HEPES 250 mM, sorbitol 330 mM, EDTA 0.5 M, β-mercaptoethanol 5m M, pH 7.6) and finally resuspended in different buffers depending on the further analysis (fig.1).

2.1.2. Analysis of chlorophyll and carotenoids

The content in chlorophyll and carotenoids of different plastid suspensions were evaluated as described by Bonora et al. (2000) with some modifications. Plastid pellets were resupended in cold absolute ethanol, purified through an octadecyl silica cartridge (Waters C-18 Sep-Pack) and eluted with a few milliliters of ethyl acetate. The solvents were removed on a rotator evaporator (DNA-mini) at 30ºC and the residues were dissolved in ethanol. The solutions were filtered through a 45 µm HVLP Millipore filter. Absorption spectra of plastid extracts were recorded at room temperature, 280-720 nm range, with a DU® 640B, Beckman (USA) spectrophotometer. Chlorophyll and carotenoid contents were calculated with the following equations: total chlorophyll mg ml^{-1} = 8.02(OD643) + 20.2(OD647) and total carotenoid mg ml^{-1} = (OD450)/0.25 (Fray and Grierson, 1993). Three independent biological replicates were measured for each developmental plastid stage suspension considered.

2.1.3. Confocal laser microscopy

A Leica TCS SP2 laser confocal microscope (Leica Microsystem Heidelberg GmbH) was used for plastid visualization after the resuspension in HEPES 250 mM, sorbitol 330 mM, EDTA 0.5 M, pH 7.6 buffer. The carotenoids and the chlorophyll were excited using the 488 nm line from the argon laser, and the emitted light was collected in separate channels, at wavelengths between 500 and 510 nm, respectively between 740 and 750 nm,. Transmitted light was also collected in a separate channel. Emission spectra of plastids was corrected by using the spectral mode of Leica Confocal software. The carotenoid and chlorophyll autofluorescence were false-colored green and red, respectively. Three independent biological replicates were observed corresponding to each developmental plastidial stage and at least 20 images were measured for each one. Each plastid (at least 50 plastids were analysed) was treated as an independent data point to calculate the average fluorescence emission spectra for each developmental plastidial stage.

2.1.4. Determination of plastid integrity

The intactness of plastids was determined using the fluorescent dye carboxylfluorescin diacetate (Shulz et al., 2004). Plastids were resuspended in a bicine 25 mM, Hepes 25 mM, pH 9, magnesium

chloride ($MgCl_2$) 2 mM, dithiothreitol (DTT) 2mM, sorbitol 0.4 M buffer, and equilibrated by a 5 min incubation with an equal volume of carboxyfluorescin diacetate (CDFA; Molecular Probesm Leiden, The Netherlands), final concentration 0.0025% w/v. Plastids suspension were examined with a WF LEICA Inv N1 microscope, (excitation filter BP 455–490, beam splitter FT 510, and emission filter, either "fluorescein-specific" BP 520–560 or "nonspecific"LP 520)The images were captured with a digital camera (Leica DL500) attached to the magnifying lens. Three independent biological replicates were observed for each developmental plastid stage suspension considered and at least 50 images were measured in each one. The number of total plastids per milliliter in the samples was determined using a hemocytometer (Neubauer Double, Zuzi).

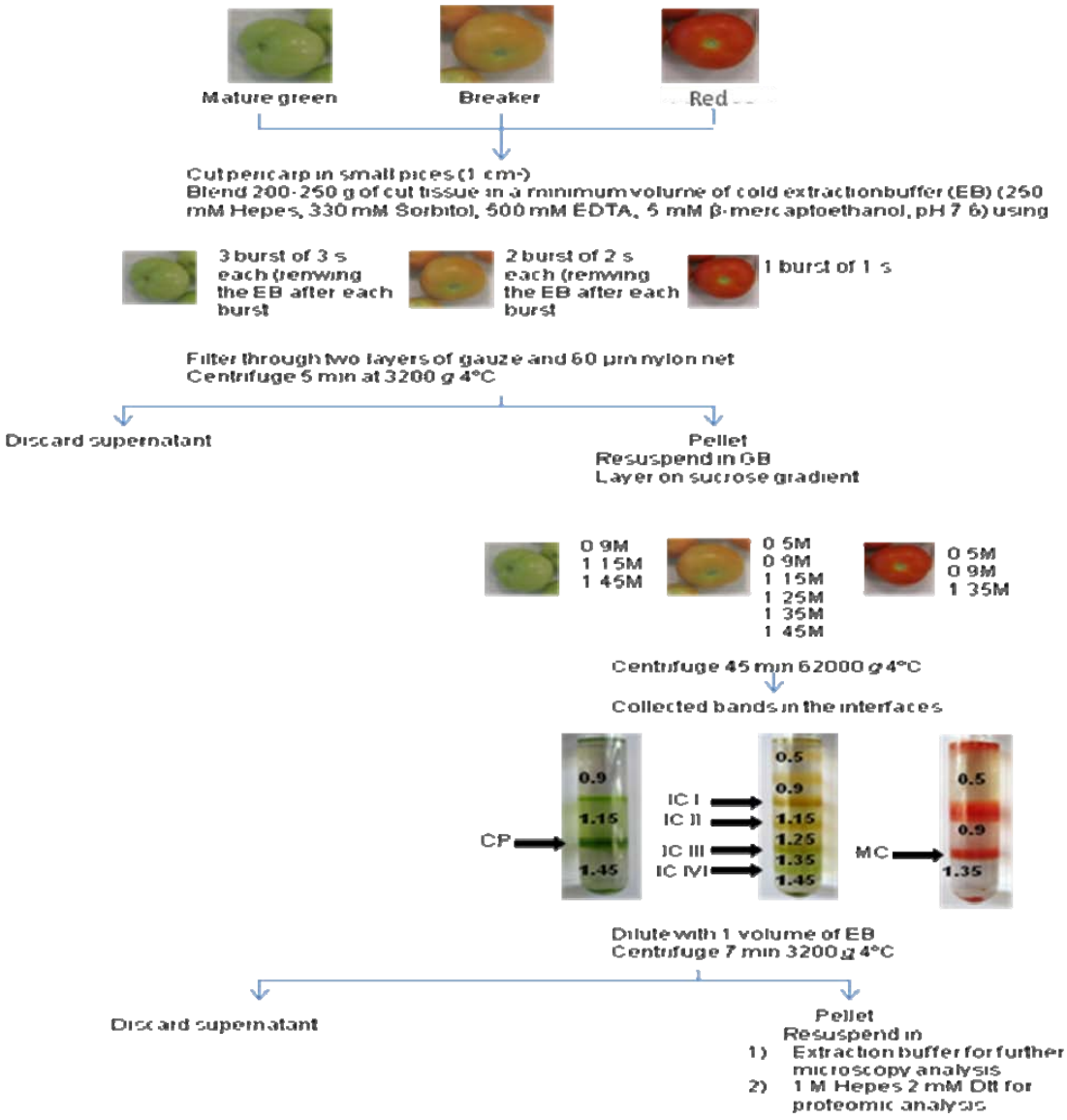


Figure 1. Method of isolation of plastids from mature green, breaker and red tomato fruits. The working temperature is 4ºC.

3. Results and Discussion

3.1. Isolation and purification of plastids in different development stages

In order to make a proteomic or/and metabolomic approach of the chloroplast to chromoplast transition in tomato fruit, it is compulsory to isolate not only chloroplasts and chromoplasts but also intermediate forms (immature chromoplast). The proteome description of this intermediary structure could help us elucidate the regulatory networks that control this complex process, probably active for a short period of time, and perhaps detectable only at the beginning of the transition. There is a lot of literature available on the isolation of both chloroplasts and chromoplasts from leaves and fruit pericarp, respectively. The most popular method is the differential and density gradient centrifugation, most frequently performed in percoll and sucrose gradients (Josse et al., 2000; Siddique et al., 2006; van Wijk et al., 2007; Martí et al., 2009). Chloroplasts (from mature green stage) and immature chromoplast (from breaker stage) have never been isolated before from tomato fruit. During tomato fruit ripening the density of plastids alter and the acidity of the whole fruit increases. The low pH of red tomato (approx. 4) decreases the solubility of pectin when the pericarp is blended in the extraction buffer. Moreover, as a result of cell-wall degradation while ripening the fruit increases its content in pectins, which precipitate along with the plastids forming a gelatinous pellet. This makes the subsequent resuspension plastidial pellet difficult and the plastids become more susceptible to breaking during the next steps of the isolation. In order to prevent the pectin precipitation the ionic strength of extraction buffer was considerably increased by adding of 250 mM of Hepes. Previously used in lower concentrations (par example: 50 mM Hepes, 30 mM Mops-KOH, 50 mM Tris) (Siddique et al., 2006; van Wijk et al., 2007; Martí et al., 2008).

The gradient concentrations are different in order to adjust to the densities that vary from chloroplasts to chromoplasts (Hadjeb et al., 1988). After centrifugation, the gradient tube contains two or three prominent pigmented bands for chloroplast and chromoplast, respectively. The relative amount and the purity of the plastids from each fraction of the gradient can be assessed with a variety of techniques: 1) Analysis of the ribosomal (r)RNA profile of SDS-treated plastid bands (Bathgate et al., 1985); 2) analysis by refringence in phase contrast microscopy of different bands (Hadjeb et al., 1988); 3) identification of the major protein constituents in each band by shotgun MS/MS, assigning the proteins to their subcellular location on the basis of targeting predictions and literature data (Siddique et al., 2006); 4) determination of the main activity peaks of marker enzymes like, NADP-dependent glyceraldehydes 3-phosphate dehydrogenase (GAPDH) for plastids, cytochrome-*c* oxidase (CCO) for mitochondria, catalase (CAT) for peroxisomes, lactate dehydrogenase (LACDH)

for cytoplasm (Martí et al., 2009); 5) western blot analysis by using polyclonal antibodies against various organelle markers.

In general, all the bands of the gradient contain plastids (Bathgate et al., 1985; Siddique et al., 2006; Barsan et al., 2010), but the choice of the band is made by the contamination degree and the enrichment in plastids. It is the case of the lower bands, at the 1.15-1.45 M interface for green tomato and 0.9-1.35 M for red tomatos. The isolation of intermediary forms (from breaker stage) implies some extra complications. The color change in tomato is not uniform, this means that the chloroplast to chromoplast transition does not occur synchronously for all the organelles in the fruit. Thus, when isolating plastids from breaker tomatoes, using either the specific-chloroplast gradient or the specific-chromoplast one, we obtain a heterogeneous population of plastids at different stages of development varying from chloroplasts to chromoplasts. A more sensitive discontinuous sucrose gradient was developed, taking into account the fact that the densities of the different plastidial forms that arise during the chloroplast to chromoplast transition tend to decrease. Taking into account this phenomenon, we have designed a new gradient (see material and methods). After centrifugation, we recovered four prominent pigmented bands. To check whether the different bands of the gradient contained plastids at different stages of development, one of the first tests performed was to calculate the chlorophyll / carotenoids ratio in each band and compare it with the ratios displayed by chloroplasts (CP) - 7.04 and mature chromoplasts (MC)- 0.02 fractions (fig.2). In the breaker gradient the molar ratio the decreased from the lower to the higher bands, from 6.24 to 1.83 for ICIV and ICI, respectively.

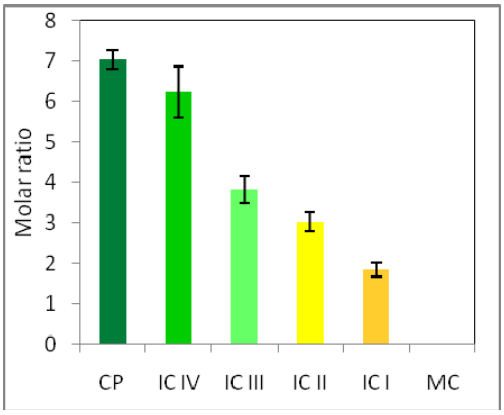


Figure 2. Chlorophyll / carotenoids molar ratio of chloroplast (CP), mature chromoplast (MC) and different development stages of immature chromoplast (IC I, IC II, IC III, IC IV) isolated from mature green, red and turning tomatoes, respectively. Vertical bars represent standard deviation values of the three independent populations.

The most conspicuous feature in the chloroplast-chromoplast transition is the accumulation of a wide variety of carotene bodies (in tomato fruit mainly lycopene), accompanied by chlorophyll

degradation. It is therefore possible that the decrease in ratio was caused by differential display of the plastids in the gradient: from more mature forms (higher bands) to more immature forms (lower bands). However, these differences in the chlorophyll / carotenoid ratio may also be driven by the presence of a different proportion of chloroplasts and chromoplasts in each band, rather than be the result of different proportions of pigments in the same organelle. To clarify this question, each plastidial fraction was analyzed by confocal microscopy.

3.2. Characterization of plastid populations by confocal microscopy

Several studies have described the use of the confocal microscopy coupled with the plastid-located green fluorescent protein (GFP) to provide significant insights into the morphology of plastid differentiation and chromoplast accumulation (Waters et al., 2004; Forth and Pyke, 2006). This technology is very useful for locating the plastids within the cell. However, for an isolated plastid population we have shown that it is possible to identify organelles in different development stages, exploiting the chlorophyll and carotenoid autofluorescence emitted at wavelengths between 500 and 510 nm (green), and between 740 and 750 nm (red), respectively, when they are excited using the 488 nm line from the argon laser. In non-fragmented cells, the confirmation that the fluorescent spots correspond to chlorophyll or carotenoid emissions is more difficult to achieve, due to the other cellular components that could have a similar fluorescence emission pattern.

The chloroplastic fraction (CP) obtained from green tomatoes appeared under the confocal microscope as a homogeneous red-emitting fluorescence population (fig. 3A). The red fluorescence is emitted by the chlorophyll. The concentration of carotenoids present in this developmental stage is low and for this reason their green fluorescence emission is masked by the red one. Isolated chromoplast suspension (MC) also appeared as a homogeneous population, but in this case it emits a green fluorescence (fig. 3E) due to the high carotenoid concentration. Chromoplast do not emitt green fluorescence because the chlorophyll is not present at this development stage of the plastid. By increasing the magnifications in the case of some isolated plastid it was possible to observe the distribution of pigments within the organelle. In chloroplasts, fluorescence spots form stacked and elongated structures (fig. 3 B), which correspond well with the arrangement of thylakoids (where chlorophyll synthesis occurres). In chromoplasts, the fluorescence spots appear as small rounded bodies (fig.3F), similar to the plastoglobuli structure where carotenid accumulate. All the four bands of the breaker gradient (ICI, ICII, ICIII, ICIV) contained a mixture of plastids at different development stages (fig.3 C, D). The fluorescence spots of these bands had different intensities of red, orange, yellow and green, caused by the different chlorophyll / carotenoid ratio of each organelle. In order to find out the percentage of chloroplasts, immature chromoplasts and mature

chromoplasts of each band of the breaker gradient, three independent biological replicates were observed and at least 50 images were measured in each one. The lower bands, ICIV (1.45-1.35M) and ICIII (1-35-1.25M), showed a high chloroplastic contamination (between 30-50%). The chloroplastic contamination remained high (> 20%) in the ICII band (1.25-1.15 m), but its level was acceptable (> 10%) in the ICI band (0.9-1.15 M). Chromoplast contamination is present in higher bands, IC I and IC II, but at non disruptive levels (< 5%). Based on these results, the IC I (0.9-1.15 M) band was considered as the most representative for the immature chromoplast population with a total contamination by other plastids lower than 15%.

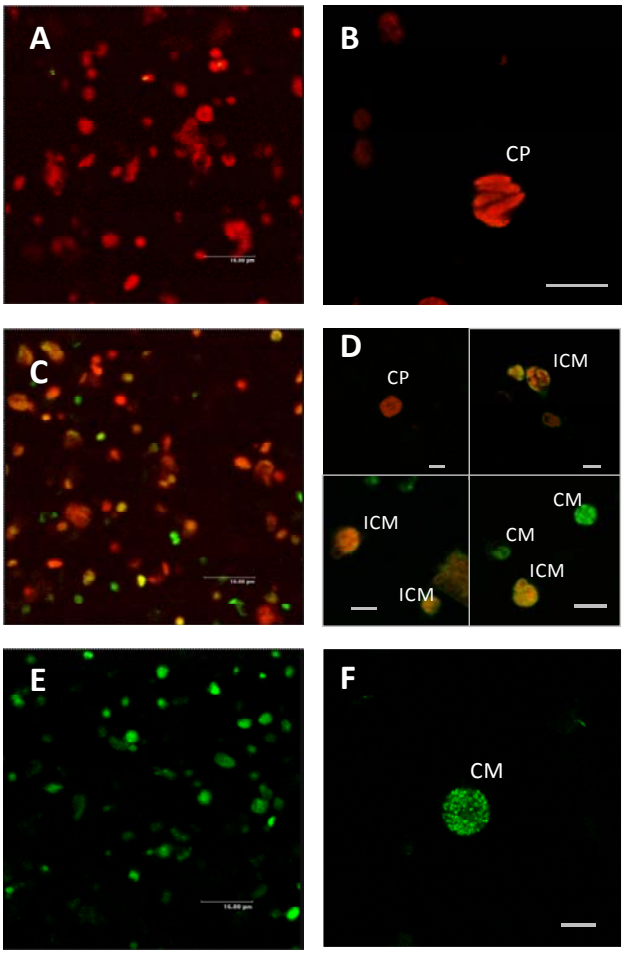


Figure 3. Confocal images of chloroplasts (A-B), immature chromoplasts (C-D) and mature chromoplast (E-F) of analyzed plastids suspensions isolated from mature green, breaker and red tomatoes. Images are overlays of chlorophyll fluorescence and carotenoid fluorescence, the structures containing mainly chlorophyll appear red, those containing only carotenoid appear green and those containing both chlorophyll and carotenoids appear orangey red/yellow. Scale bars=16 µm (A, C, E), 8 µm (B, F), 4 µm (D)

The emission spectra of the selected enriched chloroplast, immature chromoplast and mature chromoplast fractions was calculated taking advantages of the spectral mode of the Leica Confocal software (fig.4). The spectral profiles of the fractions reflected the different developmental stages of

the plastids. The chloroplastic fraction presents high levels of chlorophyll, showing a peak of fluorescence emission at 683.5 nm, and only trace amounts of carotenoids. Immature chromoplast showed a chlorophyll degradation of coupled with a modest increase in carotenoids. Finally, the chromoplastic suspension was characterized by the complete disappearance of chlorophyll and a substantial increase of total carotenoids with a spectral profile characterized by two peaks: one at 529.6 nm (β-carotene) and the other at 548.1 m (lycopene). The standard deviation of the emission spectral curve of immature chromoplast fraction is higher than the rest. This means that all immature chromoplast are not in the same developmental stage.

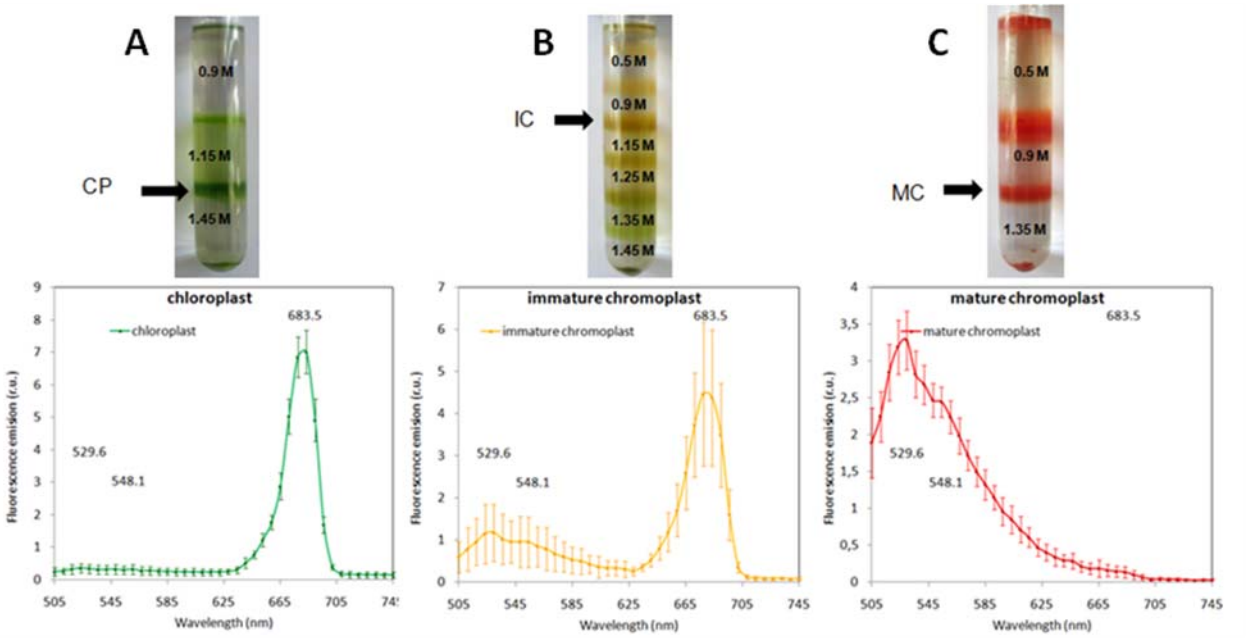


Figure 4. Fluorescence emission spectra of different stages of chloroplast-chromoplast transition: **A)** chloroplasts, **B)** Immature chromoplasts, **C)** mature chromoplasts. The numbers represent the maximum fluorescence positions. The spectra were normalized at their mean. Excitation wavelength: 408 nm. Vertical bars represent standard deviation values (n > 50).

3.3. Determination of plastids integrity

Another important parameter in determining the quality of plastid purification is the analyzis of the degree of integrity after isolation. There are different methods that can be used in this purpose. Intact plastids can be distinguished from those stripped of their bounding membrane by their opaque appearance using a phase-contrast microscope (Bathgate et al., 1985). Intact chloroplasts are surrounded by a more pronounced halo compared with the broken ones. One drawback of this method is that chloroplast envelopes can break, releasing stromal content, and then reseal, retaining

the appearance of intactness (Walker et al., 1987). Another commonly used technique consists in measuring the activity of the marker enzyme NADP-dependent glyceraldehyde 3-phosphate dehydorgenase (Bathgate et al., 1985). Recently, another method has been developed by Schulz et al. (2004), which uses the fluorescent dye carboxyfluorescin diacetate (CFDA). CFDA fluoresces strongly when de-esterified to carboxyfluorescin (CF). Up to three *Arabidopsis* carboxylsterases are predicted to be targeted to the chloroplast stroma (Emanuelsson et al., 2000). We have used this technique to determine the integrity degree of the plastidial fractions. Figure 5 shows images of a chloroplast suspension isolated from green tomato and incubated with CFDA analyzed by fluorescence microscopy. Chloroplasts that had taken up CFDA fluoresced intensely green, while chloroplasts that did not take up CFDA were red (fig.5b).

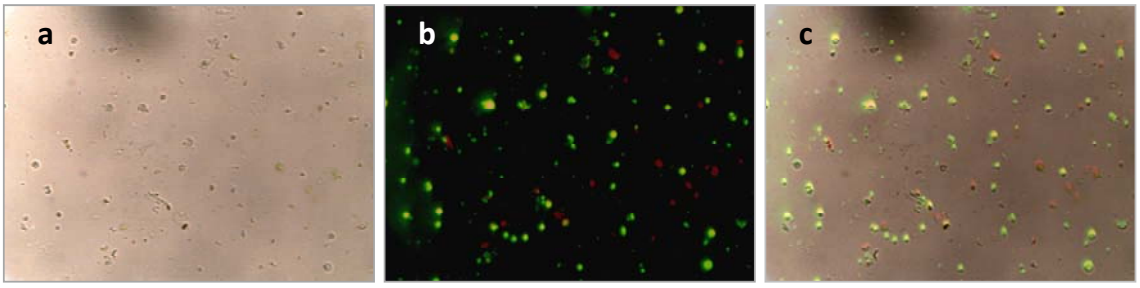


Figure 5. Chloroplast suspension isolated from green tomato and incubated with CFDA. (a) Chloroplast suspension viewed with brightlfield objective, (b) nonspecific wavelengths filter to monitor CFDA and chlorophyll fluorescence, and (c) and overlay of brightfield and fluorescence images

This method can be successfully used to determine the integrity of the immature chromoplast fractions, but is restrictive for the chromoplast suspensions due to the absence of red fluorescence in the chromoplasts due to theit lack in chlorophyll. To solve this problem, we used an overlay of brightfield and fluorescence images to calculate the percentage of intact plastids in each fraction. Three independent biological replicates were observed and at least 50 images were measured in each plastidial fraction, obtaining a percentage of intactness of chloroplasts, immature chromoplasts, and mature chromoplasts between 85-90, 80-85 and 65-70 %, respectively.

GENERAL CONCLUSION

The data have been largely discussed in each chapter. We now offer a general conclusion on our work.

We have employed high throughput technologies for studying the structure and function of plastids. Analysis of the proteome of red fruit chromoplasts revealed the presence of 988 proteins corresponding to 802 Arabidopsis unigenes, among which 209 had not been listed so far in plastidial data banks. These data revealed several features of the chromoplast. Surprisingly, chromoplasts contain the entire set of Calvin cycle proteins including Rubisco, as well as the oxidative pentose phosphate pathway (OxPPP). The low number of proteins involved in photosynthesis, with only 22% and 39% of the proteins of PSI and PSII respectively associated with the presence in the chromoplast of active chlorophyll catabolism and autophagy of photosynthetic proteins is a clear indication of the disruption of the photosynthesis. Chromoplasts lacked proteins of the chlorophyll biosynthesis branch and contained proteins involved in chlorophyll degradation Proteins of lipid metabolism and trafficking were well represented. Key proteins for the synthesis of phospholipids, glycolipids and sterols were identified along with some proteins involved in the lipoxygenase (LOX) pathway required for the synthesis of lipid-derived aroma volatiles. They have been described in the chloroplast and they lead to the formation oxylipins, which are important compounds for plant defense responses. Proteins involved in starch synthesis co-existed with several starch-degrading proteins and starch excess proteins. Starch is degraded during the chloroplast to chromoplast transition to provide carbon and energy necessary to sustain the metabolic activity during fruit ripening. None of the proteins involved in the thylakoid transport machinery were discovered.

The availability of proteomic data of tomato chromoplast and expression data of a wide range of tomato genes (The Tomato Expression Database: http://ted.bti.cornell.edu) allowed classifying genes encoding chromoplastic proteins according to their expression pattern. We analyzed 87 unigenes whose encoded proteins are located in the chromoplast into five evolution profiles. These helped us confirm the data issued from the analysis of the chromoplast proteome: it showed a down regulation of the genes involved in the photosynthesis and an increase in the expression of the genes encoding proteins involved in Calvin cycle, lipid, starch biosynthesis and degradation and stress response. Interestingly genes involved in aroma production such as *ADH* or *LOXC* had a constant increase in gene expression. This could be related to the increase in aroma production via the LOX pathway.

About 1200 proteins were discarded in the study of the chromoplastic proteome on the basis of their absence from plastidial databases or the absence of the peptide signal. The availability of full

genome sequences of plants has revealed the extent and range of plastid-contained proteins. Algorithms have been developed based on known properties of these signals and further refined on experimentally determined sequences to identify transit peptides, the most faithful to date being TargetP (http://www.cbs.dtu.dk/services/TargetP/). However, genomic and transcriptomic technologies are not very useful to make assumptions about protein localization, especially in the case of protein without signal sequences. To test their location we have chosen a pool of 9 proteins and we have visualized their location after coupling them with GFP in the single-cell system. The results did not confirm their plastidial location with the exception of one protein.

The nest step forward is the analysis of the chloroplast to chromoplast transition. Despite numerous studies, our knowledge of the regulatory networks underlying chloroplast to chromoplast differentiation in the fruit is surprisingly limited. Today several tools are available and their correct use and application may be useful for elucidate the cell biology of the chromoplast differentiation. In this sense, genomics and transcriptomics approaches are very useful for elucidating the processes that take place in a cell or in an organelle, allowing gene sequencing and quantifying the level of expression of these, using techniques that analyze thousands of molecules of mRNA. Actually, it is known that plastid genome (discussed below) encodes less than 80 proteins, the rest of proteins required for the variety of plastids functions, are encoded nuclear proteins that are translated in the cytoplasm and imported into the plastids. The targeting signal is localized at the N-terminus of the proteins as a transit peptide or signal sequence (Soll, 2002). There is no previous data on fruit chromoplast proteomics, neither on the isolation of immature chromoplast. We report here a protocol for the isolation of plastids in different developmental stages as well as a characterization by confocal microscopy and a determination on plastid integrity.

REFERENCES

.Bramley, P.M. (2002). Regulation of carotenoid formation during tomato fruit formation and development. Journal of Experimetal Botany. 53: 2107-2113.

Abbott, J. A., Saftner, R. A., Camp, M. J., Stommel, J. (2005). Sensory and objective quality attributes of beta-carotene and lycopene-rich tomato fruit. Journal of the American Society for Horticultural Science. 130: 244-251.

Abdallah, F., Salamini, F, Leister, D.(2000). A prediction of the size and evolutionary origin of theproteome of chloroplasts of Arabidopsis. Trends in Plant Science. 5: 141-142.

Abel, S., Nguyen, M. D., Chow, W., Theologis, A. (1995). ACS4, a primary indoleacetic acid-responsive gene encoding 1-aminocyclopropane-1- carboxylate synthasein Arabidopsis thaliana: structural, characterization, expression in Escherichia coli,and expression characteristics in response to auxin. Journal of Biological Chemistry. 270: 19093–19099.

Ahrazem, O., Rubio-Moraga, A., Castillo López, R. and Gómez-Gómez, L. (2010). The expression of a chromoplast-specific lycopene beta cyclase gene is involved in the high production of saffron's apocarotenoid precursors. Journal of Experimetal Botany. 61: 105-119.

Alba, R., Cordonnier-Pratt, M.M., Pratt, L.H. (2000). Fruit-localized phytochromes regulate lycopene accumulation independently of ethylene production in tomato. Plant Physiology. 123:363–370.

Alba, R., Fei, Z, Payton, P., Liu, Y., Moore, S.L., Debbie, P., Gordon, J.S., Rose, J.K.C., Martin, G., Tanksley, S.D., Bouzayen, M., Jahn, M.M. and Giovannoni, J. (2004). ESTs, cDNA microarrays, and gene expression profiling: tools for dissecting Plant Physiology and development.. The Plant Journal. 39: 697–714.

Alos, E., Cercos, M., Rodrogo, M.J., Zacarias, L. and Talon, M. (2006). Regulation of color break in citrus fruit. Changes in pigment profiling and gene expression induced by gibberellins and nitrate, two ripening retardants. Journal of Agricultural and Food Chemistry. 54: 4888-4895.

Alquezar, B., Zacarías, L. and Rodrigo, M.J. (2010) Molecular and functional characterization of a novel chromoplast-specific lycopene β-cyclase from Citrus and its relation to lycopene accumulation. Journal of Experimetal Botany. 60: 1783-1797.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. (1990). Basic local alignment searchtool. Journal of Molecular Biology 215, 403-410.

Andersson MX, Goksor M, Sandelius AS. (2007). Optical manipulation reveals strong attractingforces at membrane contact sites between endoplasmic reticulum and chloroplasts. Journal ofBiological Chemistry 282, 1170-1174.

Andersson, M.X., Sandelius, A.S. (2004). A chloroplast-localized vesicular transport system: a bio-informatics approach. BMC Genomics. 5, 40, doi:10.1186/1471-2164-5-40.

Andon, N.L., Hollingworth, S., Koller, A., Greenland, A.J., Yates 3rd Jr, Haynes, P.A. (2002). Proteomic characterization of wheat amyloplasts using identification of proteins by tandem mass spectrometry. Proteomics. 2: 1156-1168.

Aoki, K., Yamamoto, M., Wada, K. (1998). Photosynthetic and heterotrophic ferredoxin isoproteins are colocalized in fruit plastids of tomato. Plant Physiology.118: 439-449.

Arango, Y. and Heise, K.P. (1998). Localization of a-tocopherol synthesis in chromoplast envelope membranes of Capsicum annuum L. fruits. Journal of Experimetal Botany. 49: 1259–1262.

Austin, J.R., Frost, E., Vidi, P., Kessler, F. and Staehelin, L.A. (2006). Plastoglobules are lipoproteins subcompartments of the chloroplast that are permanently coupled to thylakoid membranes and contain biosynthetic enzymes. Plant Cell. 18: 1693-1703.

Awai, K., Xu, C., Tamot, B., Benning, C. (2006). A phosphatidic acid-binding protein of thechloroplast inner envelope membrane involved in lipid trafficking. Proceedings of the National Academy of Sciences, USA. 103: 10817-10822.

Ayub, R., Guis, M., Ben Amor, M., Gillot, L., Roustan, J.P., Latche, A., Bouzayen, M. and Pech, J. C. (1996). Expression of ACC oxidase antisense gene inhibits ripening of cantaloupe melon fruits. Nature Biotechnology.14:862–866.

Baginsky, S., Gruissem, W. (2004). Chloroplast proteomics: potentials and challenges. Journal of Experimental Botany. 55: 1213-1220.

Baginsky, S., Siddique, A., Gruissem, W. (2004). Proteome analysis of tobacco bright yellow-2 (BY-2) cell culture plastids as a model for undifferentiated heterotrophic plastids. Journal of Proteome Research. 3: 1128-1137.

Baldwin, E.A., Scott, J.W., Shewmaker, C.K., Schuch, W. (2000). Flavor trivia and tomato aroma: biochemistry and possible mechanisms for control of important aroma components. HortScience. 35:1013–1022.

Balmer, Y., Vensel, W.H., DuPont, F.M., Buchanan, B.B., Hurkman, W.J. (2006). Proteome of amyloplasts isolated from developing wheat endosperm presents evidence of broad metabolic capability. Journal of Experimental Botany. 57: 1591-1602.

Barry, C.S. and Giovannoni, J.J. (2006). Ripening in the tomato Green-ripe mutant is inhibited by ectopic expression of a protein that disrupts ethylene signaling. Proceeding of the National Academy of Sciences of the United States of America. 103:7923–7928

Barry, C.S., Llop-Tous, M.I., Grierson, D. (2000). The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato. Plant Physiology.123:979–986.

Barry, C.S., McQuinn, R.P., Chung, M.Y., Besuden, A. and Giovannoni, J.J. (2008). Amino acid substitutions in homologs of the STAY-GREEN protein are responsible for the green-flesh and chlorophyll retainer mutations of tomato and pepper. Plant Physiology.147: 179-187.

Barsan, C., Sanchez-Bel, P., Rombaldi, C., Egea, I., Rossignol, M., Kuntz, M., Zouine, M., Latché, A., Bouzayen, M. and Pech, J.C. (2010). Characteristics of the tomato chromoplast proteome revealed by proteomic analysis. Journal of Experimetal Botany. 61: 2413-2431.

Bathgate, B., Purton, M.E., Grierson, D. and Goodenough, P.W. (1985) Plastid changes during the conversion of chloroplasts to chromoplasts in ripening tomatoes. Planta. 165: 197-204.

Bedhomme, M., Hoffmann, M., McCarthy, E.A., Gambonnet, B., Moran, R.G., Rébeillé, F.,Ravanel, S. (2005). Folate metabolism in plants: an Arabidopsis homolog of the mammalianmitochondrialfolate transporter mediates folate import into chloroplasts. Journal of BiologicalChemistry. 280: 34823-34831.

Ben-Shaul, Y. and Klein, S. (1965). Development and structure of carotene bodies in carrot roots. Botan. Gaz.126: 79-85.

Ben-Shaul, Y., Treffry, T., and Klein, S. (1968). Fine Structure Studies of Carotene Body Development, J. Microsc. 7:265–274.

Bernhardt, A., Lechner, E., Hano, P., Schade, V., Dieterle, M., Anders, M., Jody Dubin, M., Benvenuto, G., Bowler, C., Genschik, P. and Hellmann, H. (2006). CUL4 associates with DDB1 and DET1 and its downregulation affects diverse aspects of development in Arabidopsis thaliana. The Plant Journal. 47:591-603.

Biale, J.B. (1964). Growth, maturation, and senescence in fruits: recent knowledge on growth regulation and on biological oxidations has been applied to studies with fruits. Science. 146:880–888.

Birtic, S., Ginies, C., Causse, M., Renard, C.G.C., Page, D. (2009). Changes in volatiles and glycosidesduring fruit maturation of two contrasted tomato (Solanum lycopersicum) lines. Journal ofAgricultural and Food Chemistry. 57: 591–598.

Blas, A.L., Ming, R., Liu, Z., Veatch, O.J., Paull, R.E., Moore, P.H. and Yu, Q. (2010). Cloning of papaya chromoplast specific lycopene β-cyclase, CpCYC-b, controlling fruit flesh color

reveals conserved microsynteny and a recombination hotspot. Plant Physiology.152: 2013-2022.

Bonora, A., Pancaldi, S., Gualandri, R. and Fasulo, M.P. (2000). Carotenoid and ultrastructure variations in plastids of Arum italicum Miller fruit during maturation and ripening. Journal of Experimetal Botany. 51: 873-884.

Bouvier, F. and Camara, B. (2007). The role of plastids in ripening fruits. In The structure and functions of plastids. Edited by Wise, R.R. and Hoober, J.K. pp. 419-432. Springer, Dordrecht.

Bouvier, F., Backhaus, A., Camara B. (1998). Induction and control of chromoplast-specificcarotenoid genes by oxidative stress. Journal of Biological Chemistry. 46: 30651-30659.

Bouyssié, D., Peredo, A.G., Mouton, E., Albigot, E., Roussel, D., Ortega, N., Cayrol, C., Burlet-Schiltz, O., Girard, J.P., Monsarrat, B. (2007). Mascot file parsing and quantification (MFPaQ),a new software to parse, validate, and quantify proteomics data generated by ICAT andSILAC mass spectrometric analyses: Application to the proteomics study of membraneproteins from primary human endothelial cells. Molecular & Cell Proteomics. 6: 1621-1637.

Bravdo, B.M., Palgi, A., Lurie, S. (1977). Changing ribulose diphosphate carboxylase/oxygenaseactivity in ripening tomato fruit. Plant Physiology. 60: 309-312.

Bréhélin, C. and Kessler, F. (2008) The plastoglobule: a bag full of lipid biochemistry tricks. Photochemistry and Photobiology. 84: 1388-1394.

Briat, J.F., Gigot, C., Laulhère, J., Mache, R. (1982). Visualization of a spinach plastid transcriptionally active DNA-protein complex in a highly condensed structure. Plant Physiology. 69: 1205-1212.

Büker, M., Schünemann, D. and Borchert, S. (1998). Enzymic properties and capacities of developing tomato (Lycopersicon esculentum L.) fruit plastids. Journal of Experimetal Botany. 49: 681-691.

Buttery, R.G. (1993). Quantitative and sensory aspects of flavor of tomato and other vegetables and fruits. In: Acree TE, Teranishi R, editors. Flavor Science: Sensible Principles and Techniques. American Chemistry Society, Washington, DC. 259–286.

Camara, B., Bardat, F., Dogbo, O., Brangeon, J., Moneger, R. (1983). Terpenoid metabolism in plastids. Isolation and biochemical characteristics of Capsicum annuum chromoplasts. Plant Physiology. 73: 94–99.

Camara, B., Hugueney, P., Bouvier, F., Kuntz, M. and Monéger, R. (1995). Biochemistry and molecular biology of chromoplast development. International Review of Cytology163: 175–247.

Campbell, D.A., Cockshutt, A.M., Porankiewicz-Asplund, J. (2003). Analysing photosynthetic complexes in uncharacterized species or mixed microalgal communities using globalantibodies. Physiologia Plantarum. 119: 322-327.

Canoles, M.A., Beaudry, R.M., Li, C.Y., Howe, G. (2006). Deficiency of linolenic acid in Lefad7 mutant tomato changes the volatile profile and sensory perception of disrupted leaf and fruit tissue. Journal of the American Society for Horticultural Science. 131:284–289.

Carrara, S., Pardossi, A., Soldatini, G.F., Tognoni, F., Guidi, L. (2001). Photosynthetic activity of ripening tomato fruit, Photosynthetica. 39:75-78

Cerutti, H, Osman, M, Grandoni, P, Jagendorf, AT. (1992). A homolog of Escherichia coli RecA protein in plastids of higher plants. Proceedings of the National Academy of Sciences, USA 89: 8068–8072

Chen, D.M., Xue, Y., Liu, J.M., Wang, Y.J. and Chen, H. (2001) Isolation of lycopene beta-cyclase cDNA from Daucus carota and its differential expression in roots. Acta Botanica Sinica. 43: 1265-1270

Chen, G., Hackett, R., Walker, D., Taylor, A., Lin, Z., Grierson, D. (2004). Identification of a specific isoform of tomato lipoxygenase (TomloxC) involved in the generation of fatty acid-derived flavor compounds. Plant Physiology. 136:2641–2651.

Chervin, C., El-Kereamy, A., Roustan, J.P., Latche,´ A., Lamon, J., Bouzayen, M. (2004). Ethylene seems required for the berry development and ripening in grape, a non-climacteric fruit. Plant Scienceence. 167:1301–1305.

Cheung, A.Y., McNellis, T. and Piekos, B. (1993) Maintenance of chloroplast components during chromoplast differentiation in the tomato mutant green flesh. Plant Physiology.101: 1223-1229.

Chew, O., Wheland, J., Millar, A.H. (2003). Molecular definition of the ascorbate-glutathione cycle inArabidopsis mitochondria reveals dual targeting of antioxidant defenses in plants. Journal of BiologicalChemistry. 278: 46869-46877.

Clément, C., Mischler, P., Burrus, M. and Audran, J.C. (1997). Characteristics of the photosynthetic apparatus and CO_2 fixation in the flower bud of Lilium I. corolla. The International Journal of Plant Science.158: 794-800.

Cookson, P.J., Kiano, J., Fraser, P.D., Romer, S., Shipton, C.A., Schuch, W., Bramley, P.M. and Pyke, K.A. (2003). Increases in cell elongation, plastid compartment size and translational

control of carotenoid gene expression underly the phenotype of the high pigment-1 mutant of tomato. Planta. 217: 896-903.

Corona, V., Aracri, B., Kosturkova, G., Bartley, G.E., Pitto, L., Giorgetti, L., Scolnik, P.A., Giuliano, G. (1996). Regulation of a carotenoid biosynthesis gene promoter during plant development. The Plant Journal. 9:505–512.

Cosgrove, D.J. (1989). Characterization of long-term extension of isolated cell walls from growing cucumber hypocotyls. Planta. 177: 121-130.

Cosgrove, D.J. (1997). Assembly and enlargement of the primary cell wall in plants. Annual Review of Cell and Developmental Biology. 13: 171-201.

Croteau, R. and Karp, F. (1991). Origin of natural odorants. In Perfumes. Art, Science and Technology; Muller, P.M., Lamparsky, D., Eds.; Elsevier Applied Science. London, England, pp.101-126.

Cunningham, F.and Gantt, E. (1998). Genes and enzymes of carotenoid biosynthesis in plants. Annual Review of Plant Physiology and Plant Molecular Biology. 49:557–583.

Dalal, M., Chinnusamy, V. and Bansal, K.C. (2010). Isolation and functional characterization of Lycopene β-cyclase (CYC-B) promoter from Solanum habrochaites. BMC Plant Biology. 10: 61-76.

De Santis-Maciossek, G., Kofer, W., Bock, A., Schoch, S., Maier, R.M., Wanner, G., Rüdiger, W., Koop, H.R., Herrmann, R.G. (1999). Targeted disruption of the plastid RNA polymerase genesrpoA, B and C1: molecular biology, biochemistry and ultrastructure. The The Plant Journal. 18:477-489.

Delker, C., Zolman, B.K., Miersch, O., Wasternack, C. (2007). Jasmonate biosynthesis inArabidopsis thaliana requires peroxisomal β-oxidation enzymes- Additional proof byproperties of pex6 and aim1. Phytochemistry. 68: 1642-1650.

Deng, X.W., Gruissem, W. (1987). Control of plastid gene expression during development: thelimited role of transcriptional regulation. Cell. 49: 379-387.

Deruere, J., Romer, S., d'Harlingue, A., Backhaus, R.A., Kuntz, M. and Camara, B. (1994). Fibril assembly and carotenoid over-accumulation in chromoplasts: a model for supramolecular lipoprotein structures. Plant Cell 6: 119–133.

Devide, Z. (1970). Ultrastructural changes of plastids in ripe fruits of Cucurbita pepo cv. olifera. Acta Bot. Croatica. 29: 57-60.

Devidé, Z. and Ljubesic, N. (1974). The reversion of chromoplasts to chloroplasts in pumpkin fruits. Z. Planzenphysiol. 73: 296-306.

Drescher, A., Ruf, S., Calsa Jr, T., Carrer, H., Bock, R. (2000). The two largest chloroplast genome-encoded open reading frames of higher plants are essential genes. The The Plant Journal. 22: 97-104.

Edelman, M, Mattoo, AK. (2008). D1-protein dynamics in photosystem II: the lingering enigma. Photosynthesis Research. 98: 609-620.

El-Kafafi, E., Karamoko, M., Pignot-Paintrand, I., Grunwald, D., Mandaron, P., Lerbs-Mache S., Falconet, D. (2008). Developmentally regulated association of plastid division protein FtsZ1with thylakoid membranes in Arabidopsis thaliana. Biochemical Journal. 409: 87–94.

Emanuelsson, O., Nielsen, H., Brunak, S., von Heijne, G. (2000). Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. Journal of Molecular Biology. 300: 1005-1016.

Fahn, A. (1967). Plant anatomy. Peragamon Press. pp 439-441.

Farmaki, T., Sanmartın, M., Jimenez, P., Paneque, M., Sanz, C., Vancanneyt, G., Leon, J. and Sanchez-Serrano, J.J. (2007). Differential distribution of the lipoxygenase pathway enzymes within potato chloroplasts, Journal of Experimental Botany. 58: 555–568.

Farmer, E.E., Ryan, C.A. (1992). Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell. 4: 129-134.

Faurobert, M., Mihr, C., Bertin, N., Pawlowski, T., Negroni, L., Sommerer, N., Causse, M. (2007).Major proteome variations associated with cherry tomato pericarp development and ripening. Plant Physiology. 143: 1327-1347.

Fei, Z., Tang, X., Alba, R., Giovannoni, J. (2006). Tomato Expression Database (TED): a suite of data presentation and analysis tools. Nucleic Acids Research. 34: 766–770.

Ferro, M., Brugière, S., Salvi, D., Seigneurin-Berny, D., Court, M., Moyet, L., Ramus, C., Miras, S., Mellal, M., Le Gall, S., Kieffer-Jaquinod, S., Bruley, C., Garin, J., Joyard, J., Masselon, C. and Rolland, N. (2010). AT_CHLORO, a comprehensive chloroplast proteome database with subplastidial localization and curated information on envelope proteins. Mol. Cell. Proteomics 9: 1063-1084.

Ferro, M., Salvi, D., Brugière, S., Miras, S., Kowalski, S., Louwagie, M., Garin, J., Joyard, J., Rolland, N. (2003). Proteomics of the chloroplast enveloppe membranes from Arabidopsis thaliana. Molecular & Cell Proteomics. 2: 325-345.

Forth, D. and Pyke, K.A; (2006). The suffulta mutation in tomato reveals a novel method of plastid replication during fruit ripening. Journal of Experimetal Botany. 57: 1971-1979.

Foyer, C., Noctor, G. (2003). Redox sensing and signalling associated with reactive oxygen inchloroplasts, peroxisomes and mitochondria. Physiologia Plantarum. 119: 355–364.

Fraser, P.D., Kiano, J.W., Truesdale, M.R., Schuch, W., Bramley, P.M. (1999). Phytoene synthase-2enzyme activity in tomato does not contribute to carotenoid synythesis in ripening fruit. PlantMolecular Biology. 40: 687-698.

Fraser, P.D., Pinto, M.E.S., Holloway, D.E., Bramley, P.M. (2000). Application of high-performance liquid chromatography with photodiode array detection to the metabolic profiling of plant isoprenoids. The The Plant Journal. 24: 551-558.

Fraser, P.D., Truesdale, M.R., Bird, C.R., Schuch, W., Bramley, P.M. (1994). Carotenoid biosynthesis during tomato fruit development. Plant Physiology. 105:405-413.

Fraser, P.D. and Bramley, P.M. (2004) The biosynthesis and nutritional uses of carotenoids. Progress in Lipid Research.43: 228–265.

Fray, R.G.; Grierson, D. (1993). Identification and genetic-analysis of normal and mutant phytoene synthase genes of tomato by sequencing, complementation and co-suppression Plant Molecular Biology 22: 589-602.

Friedrich, T., Steinmüller, K., Weiss, H. (1995). The proton-pumping respiratory complex I ofbacteria and mitochondria and its homologue in chloroplasts. FEBS Letters. 367: 107-111.

Galpaz, N., Wang, Q., Menda, N., Zamir, D. and Hirschberg, J. (2008). Abscisic acid deficiency in the tomato mutant high-pigment 3 leading to increased plastid number and higher fruit lycopene content. The Plant Journal. 53: 717-730.

Galvez-Valdivieso, G. and Mullineaux, P.M. (2010). The role of reactive oxygen species in signalling from chloroplasts to the nucleus. Physiologia Plantarum.138: 430–439.

Giacomelli, L., Masi, A, Ripoll, D.R., Lee, M.J., van Wijk K.J. (2007). Arabidopsis thaliana deficientin two chloroplast ascorbate peroxidases shows accelerated light-induced necrosis when levels of cellular ascorbate are low. Plant Molecular Biology. 65: 627-644.

Giacomelli, L., Rudella, A., van Wijk, K.J. (2006). High light response of the thylakoid proteome inArabidopsis wild type and the ascorbate-deficient mutant vtc2-2. A comparative proteomicstudy. Plant Physiology. 141: 685-701.

Gigot, C., Ongena, M., Fauconnier, M.-L., Paul Wathelet, J.-P., du Jardin, P. and Thonart, P. (2010). The lipoxygenase metabolic pathway in plants: potential for industrial production of natural green leaf volatiles. BASE.14: 451-460.

Giovannoni, J.J. (2001). Molecular regulation of fruit ripening. Annual Review of Plant Physiology and Plant Molecular Biology. 52: 725–749.

Giovannoni, J.J. (2004). Genetic Regulation of Fruit Development and Ripening. The Plant Cell. 16: 170–180.

Giuliano, G. and Diretto, G. (2007). Of chromoplasts and chaperones. Trends in Plant Scienceence. 12: 529-531.

Giuliano, G., Bartley, G., Scolnik, P. (1993). Regulation of carotenoid biosynthesis during tomato development. Plant Cell. 5:379–387.

Griffiths, A., Barry, C., Alpuche-Solis, A.G., Grierson, D. (1999). Ethylene and developmental signals regulate expression of lipoxygenase genes during tomto fruit ripening. Journal of Experimental Botany. 50: 793-798.

Grilli-Caiola, M.G. and Canini, A. (2004). Ultrastructure of chromoplasts and other plastids in Crocus sativus L. (Iridaceae). Plant Biosystems. 138: 43-52.

Groen, A.J.; de Vries, S.C.; Lilley, K.S. (2008). A proteomics approach to membrane trafficking. Plant Physiology. 147: 1584-1589.

Hadjeb, N, Gounaris, I., Price, C.A. (1988). Chromoplast-specific proteins in Capsicum-annuum. Plant Physiology. 88: 42-45.

Hagio, M., Gombos, Z., Várkonyi, Z., Masamoto, K., Sato, N., Tsuzuki, M. and Wada, H. (2000). Direct evidence for requirement of phosphatidylglycerol in photosystem II of photosynthesis. Plant Physiology.124: 795–804.

Hamilton, A.J., Lycett, G.W., Grierson, D. (1990). Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. Nature. 346:284–287.

Hanson, D.A., and Ziegler, S.F. (2004). Fusion of green fluorescent protein to the C-terminus of granulysin alters its intracellular localization in comparison to the native molecule. Journal of Negative Results in BioMedicine.3,2

Harlingue (d'), A., Camara, B. (1985). Plastid enzymes of terpenoid biosynthesis. Purification andcharacterization of gamma-tocopherol methyltransferase from Capsicum chromoplasts. Journal of Biological Chemistry. 260: 15200-15203.

Harris, W.M. and Spurr, A.R. (1969). Chromoplasts of tomato fruit. II. The red tomato. American Journal of Botany. 56: 380-389.

Hatch, M. D. and Mau, S.-L.(1973). Activity, location, and role of asparate aminotransferase and alanine aminotransferase isoenzymes in leaves with C_4 pathway photosynthesis. **Archives of** Biochemistry and Biophysics. 156 :195-206

Heazlewood, J.L., Verboom, R.E., Tonti-Filippini, J., Small, I., Millar, A.H. (2007). SUBA: theArabidopsis Subcellular Database. Nucleic Acids Research. 35: D213-D218.

Herrmann, K. M. (1995). The Shikimate Pathway: Early Steps in the Biosynthesis of Aromatic Compounds. The Plant Cell. 7: 907-919.

Herrmann, K.M., Weaver, L.M. (1999). The shikimate pathway. Annual Review of PlantPhysiology and Plant Molecular Biology. 50: 473-503.

Hesse, H., Hoefgen, R. (2003). Molecular aspects of methionine biosynthesis. Trends in Plant Scienceence. 8:259-262.

Horner, H.T., Healy, R.A., Ren, G., Fritz, D., Klyne, A., Seames, C. and Thronburg, R.W. (2007). Amyloplast to chromoplast conversion in developing ornamental tobacco floral nectaries provides sugar for nectar and antioxidants for protection. American Journal of Botany. 94: 12-24.

Hudák, J., Galova, E., Zemanova, L. (2005). Plastid morphogenesis. In M Pessarakli, ed, Handbookof Photosynthesis, Ed 2, CRC Press, Boca Raton, pp 221-246I.

Huff, A. (1983). Nutritional control of regreening and degreening in citrus peel segments. Plant Physiology.73: 243-249.

Hunt, C.M., Hardison, R.C., Boyer, C.D. (1986). Restriction enzyme analysis of tomato chloroplast and chromoplast DNA. Plant Physiology. 82: 1145-1147.

Hunter, A. W., Jourdan, J., Gourdie, R. G. (2003). Fusion of GFP to the Carboxyl Terminus of Connexin43 Increases Gap Junction Size in HeLa Cells. Cell Communication and Adhesion. 10: 211-214.

Jarvis, P. (2008). Targeting of nucleus-encoded proteins to chloroplasts in plants. New Phytologist. 179: 257-285.

Jeong, S.Y., Rose, A. and Meier, I. (2003). MFP1 is a thylakoid-associated, nucleoid-binding protein with a coiled-coil structure. Nucleic Acids Research. 31: 5175-5185.

Jones, B., Frasse, P., Olmos, E., Zegzouti, H., Li, Z.G., Latché, A., Pech, J.C. and Bouzayen, M. (2002). Down-regulation of DR12, an auxin-response-factor homolog, in the tomato results in a pleiotropic phenotype including dark green and blotchy ripening fruit. The Plant Journal. 32: 603-613.

Josse, E.M., Simkin, A.J, Gaffe J, Labourne, A., Kuntz, M., Carol, P. (2000). A plastid terminal oxidase associated with carotenoid desaturation during chromoplast differentiation. Plant Physiology. 123: 1427–1436.

Joyard, J., Ferro, M., Masselon, C., Seigneurin-Berny, D., Salvi, D., Garin, J., Rolland, N. (2010). Chloroplast proteomics highlights the subcellular compartmentation of lipid metabolism, Progress in Lipid Research. 49: 128–158.

Julliard, J.H., Douce, R. (1991). Biosynthesis of the thiazole moiety of thiamine (vitamin B1) inhigher plant chloroplasts. Proceedings of the National Academy of Sciences, USA. 88: 2042-2045.

Juneau, P., Le Lay, P., Böddi, B., Samson, G. and Popovic, R. (2002). Relationship between the structural and functional changes of the photosynthetic apparatus during chloroplast-chromoplast transition in flower bud of Lilium longiflorum. Photochemistry and Photobiology. 75: 377-381.

Kahlau, S., Bock, R. (2008). Plastid transcriptomics and translatomics of tomato fruit developmentand chloroplast-to-chromoplast differentiation: chromoplast gene expression largely servesthe production of a single protein. The Plant Cell. 20: 856-874.

Kahlau, S., Aspinall, S., Gray, J.C. and Bock, R. (2006). Sequence of the tomato chloroplast DNA and evolutionary comparison of Solanaceous plastid genomes. Journal of Molecular Evolution. 63: 194-207.

Kalanon, M., McFadden, I. (2008). The chloroplast protein translocation complexes of Chlamydomonas reihardtii: a bio-informatic comparison of Toc and Tic components inplants, green algae and red algae. Genetics. 179: 95-112.

Keresztes, A., Schróth, A. (1979). Light and electron microscopic investigation of in vitro starchsynthesis in chromoplasts. Cytobios. 26: 185-191.

Kessler, F., Vidi, P.A. (2007). Plastoglobule lipid bodies: their functions in chloroplasts and their potential for applications. Advances in Biochemical Engineering/Biotechnology.107:153–172.

Kim, J.E., Rensing, K.H., Douglas, C.J. and Cheng, K.M. (2010). Chromoplast ultrastructure and estimated carotene content in root secondary phloem of different carrot varieties. Planta. 231: 549-558.

Kleffmann, T., Hirsch-Hoffmann, M., Gruissem, W., Baginsky, S. (2006). Plprot: a comprehensive proteome database for different plastid types. Plant Cell Physiology. 47:432-436.

Kleffmann, T., Russenberger, D., von Zychlinski, A., Christopher, W., Sjölander, K., Gruissem, W., Baginsky, S. (2004). The Arabidopsis thaliana chloroplast proteome reveals pathway abundance and novel functions. Current Biology 14, 354-362.

Kleine, T., Voigt, C. and Leister, D. (2009). Plastid signalling to the nucleus: messengers still lost in the mists? Trends in Genetics. 25: 185-190.

Klösgen, R.B., Molik, S., Frielingsdorf, S., Gutensohn, M., Jakob, M., Marques, J.P., Hou, B. (2004). Protein transport across the thylakoid membrane. Endocytobiosis and Cell Research. 15: 518-526.

Kobayashi, H., Ngernprasirtsiri, J. and Akazawa, T. (1990). Transcriptional regulation and DNA methylation in plastids during transitional conversion of chloroplasts to chromoplasts. The EMBO Journal. 9: 307-313.

Köhler, R.H. and Hanson, M.R. (2000). Plastid tubules of higher plants are tissue-specific and developmentally regulated. Journal of Cell Science. 113: 81-89.

Kramer, D.M., Avenson, T.J. and Edwards, G.E. (2004). Dynamic flexibility in the light reactions of photosynthesis governed by both electron and proton transfer reactions. Trends in Plant Scienceence. 9: 349–357.

Kroll, D., Meierhoff, K., Bechtold, N., Kinoshita, M., Westphal, S., Vothknecht, U.C., Soll, J., Westhoff, P. (2001). VIPP1, a nuclear gene of Arabidopsis thaliana essential for thylakoidmembrane formation. Proceedings of the National Academy of Sciences, USA. 98: 4238-4242.

Kruger, N.J., von Schaewen, A. (2003). The oxidative pentose phosphate pathway: structure andorganisation. Current Opinion in Plant Biology. 6: 236-246.

Kubo, M. and Kakimoto, T. (2000) The cytokinin-hypersensitive genes of Arabidopsis negatively regulate the cytokinin-signaling pathway for cell division and chloroplast development. The Plant Journal. 23: 385-394.

Kwok, E.Y., Hanson, M.R. (2004). Stromules and the dynamic nature of plastid morphology.Journal of Microscopy. 214, 124-137.

Laborde, J.A. and Spurr, A.R. (1973). Chromoplast ultrastructure as affected by genes controlling grana retention and carotenoids in fruits of Capsicum annuum. American Journal of Botany. 60: 736-744.

Laizet, Y., Pontier, D., Mache, R., Kuntz, M. (2004). Subfamily organization and phylogenic origin of genes encoding plastid lipid-associated proteins of the fibrillin type. Journal of Genome Science and Technology. 3: 19-28.

Lange, B., Wildung, M., McCaskill, D., Croteau, R. (1998). A family of transketolases that directs isoprenoid biosynthesis via a mevalonate-independent pathway. Proceeding of the National Academy of Sciences of the United States of America. 95:2100–2104.

Leclercq, J., Ranty, B., Sanchez-Ballesta, M. T., Li, Z., Jones, B., Jauneay, A., Pech, J. C. , Latche, A., Ranjeva, R., Bouzayen, M. (2005). Molecular and biochemical characterization of LeCRK1, a ripening associated tomato CDPK-relatedkinase. Journal of Experimental Botany. 56: 25–35.

Leech, R.M. and Pyke, K.A. (1988). Chloroplast division in higher plants with particular reference to wheat. In The Division and Segregation of Organelles Edited by Boffey, S.A. and Lloyd, D. pp. 39-61. Cambridge University Press, Cambridge, UK.

Leitner-Dagan, Y., Ovadis, M., Shklarman, E., Elad, Y., David, D.R. and Vainstein, A. (2006) Expression and functional analysis of the plastid lipid-associated protein CHRC suggest its role in chromoplastogenesis and stress. Plant Physiology.142: 233-244.

Lelièvre, J.M., Latché, A., Jones, B., Bouzayen, M., Pech, J.C. (1997). Ethylene and fruit ripening. Physiologia Plantarum. 101: 727-739.

Lewinsohn, E., Schalechet, F., Wilkinson, J., Matsui, K., Tadmor, Y., Nam, K. H., Amar, O., Lastochkin, E., Larkov, O., Ravid, U. , Hiatt, W., Gepstein, S. and Pichersky, E.(2001). Enhanced levels of the aroma and flavor compound S-linalool by metabolic engineering of the terpenoid pathway in tomato fruits. Plant Physiology.127:1256–1265.

Li, L. and van Eck, J. (2007). Metabolic engineering of carotenoid accumulation by creating a metabolic sink. Transgenic Research. 16: 581-585.

Li, L., Paolillo, D.J., Parthasarathy, M.V., DiMuzio, E.M. and Garvin, D.F. (2001). A novel gene mutation that confers abnormal patterns of β-carotene accumulation in cauliflower (Brassica oleracea var. botrytis). The Plant Journal. 26: 59-67.

Libal-Weksler, Y., Vishnevetsky, M., Ovadis, M., Itzhaki, H. and Vainstein, A. (1995). Flower-specific carotenoid accumulation in chromoplasts: molecular control of carotenoid-associated proteins. Acta Horticulturae. (ISHS). 420:32-34.

Lichtenthaler, H.K. (1969). Zur Synthese der lipophilen Plastidenchinone und Sekundarcarotinoide wahrend der Chromoplastenentwicklung. Berichte der Deutschen Botanischen Gesellschaft. 82, 483–97.

Liu, Y., Roof, S., Ye, Z., Barry, C., van Tuinen, A., Vrebalov, J., Bowler, C. and Giovannoni, J.J. (2004). Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato. Proceedings of the National Academy of Sciences USA. 101: 9897–9902.

Livne, A., Gepstein, S. (1988). Abundance of the major chloroplast polypeptides duringdevelopment and ripening of tomato fruits. Plant Physiology. 87: 239-242.

Ljubesic, N. (1977). The formation of chromoplasts in fruits of Cucurbita maxima Duch. .turbaniformis.. Botanical gazette. 13B: 286-290.

Ljubesic, N., Wrischer, M. and Device, Z. (1996). Chromoplast structures in Thunbergia flowers. Protoplasma. 193: 174-180.

Ljubesic, N., Wrischer, M. and Devise, Z. (1991). Chromoplasts-the last stages in plastid development. International Journal of Developmental Biology. 35: 251-258.

Lois, L., Rodrıguez-Concepcion, M., Gallego, F., Campos, N., Boronat, A. (2000). Carotenoid biosynthesis during tomato fruit development: regulatory role of 1-deoxy-D-xylulose 5-phosphate synthase. The Plant Journal. 22:503–513.

Lopez, A.B., van Eck, J.V., Conlin, B.J., Paolillo, D.J., O'Neill, J. and Li, L. (2008). Effect of the cauliflower Or transgene on carotenoid accumulation and chromoplast formation in transgenic potato tubers. Journal of Experimetal Botany. 59: 213-223.

Lopez-Juez, E. (2007). Plastid biogenesis, between light and shadows. Journal of Experimetal Botany. 58:11-26.

Lopez-Juez, E. and Pyke, K.A. (2005). Plastids unleashed: their development and their integration in plant development. International Journal of Developmental Biology. 49: 557-577.

Lu, S. and Li, L. (2008). Carotenoid metabolism: Biosynthesis, regulation,and beyond. Journal of Integrative Plant Biology. 50: 778–785.

Lu, S., van Eck, J., Zhou, X., Lopez, A.B., O'Halloran, D.M., Cosman, K.M., Conlin, B.J., Paolillo, D.J., Garvin, D.F., Vrebalov, J., Kochian, L.V., Küpper, H., Earle, E.D., Cao J. and Li, L. (2006). The cauliflower Or Gene encodes a DnaJ cysteine-rich domain containing protein that mediates high levels of ß-carotene accumulation. Plant Cell. 18: 34 3594-3605.

Luengwilai, K.; Beckles, D. M. (2009). Starch Granules in Tomato Fruit Show a Complex Pattern of Degradation. Journal of Agricultural and Food Chemistry. 57: 8480–8487.

Maass, D., Arango, J., Wüst, F., Beyer, P. and Welsh, R. (2009). Carotenoid crystal formation in Arabidopsis and carrot roots caused by increased phytoene synthase protein levels. PloS ONE 4: 6373.

Majid, N.A. and Parveez, G.K.A.(2006). Evaluation of Green Fluorescence Protein (GFP) as a Selectable Marker for Oil Palm Transformation via Transient Expression. Asia Pacific Journal of Molecular Biology and Biotechnology.15 :1-8.

Marano, M.R., Carrillo, N. (1992). Constitutive transcription and stable RNA accumulation inplastids during the conversion of chloroplasts to chromoplasts in ripening tomato fruits. PlantPhysiology. 100: 1103-1113.

Marano, M.R. and Carrillo, N. (1991). Chromoplast formation during tomato fruit ripening - no evidence for plastid DNA methylation. Plant Molecular Biology. 16: 11-19.

Marano, M.R., Serra, E.C., Orellano, G. and Carrillo, N. (1993). The path of chromoplast development in fruits and flowers. Plant Science. 94: 1-17.

Marti, M.C., Camejo. D., Olmos, E., Sandalio, L.M., Fernandez-Garcia, N., Jimenez, A. and Sevilla, F. (2009). Characterisation and changes in the antioxidant system of chloroplasts and chromoplasts isolated from green and mature pepper fruits. Plant Biosystems. 11: 613-624.

Martineau, B., Houck, C.M., Sheehy, E. and Hiatt, W.R. (1994). Fruit-specific expression of the A. tumefaciens isopentenyl transferase gene in tomato: effects on fruit ripening and defense-related gene expression in leaves. The Plant Journal. 5: 11-19.

Martinez-Madrid, M.C., Serrano, M., Riquelme, F., Romojaro, F. (1996). Polyamines, abscisic acidand ethylene production in tomato fruit. Phytochemistry. 43: 323-326.

Mathews, H., Clendennen, S.K., Caldwell, C.G., Liu, X.L., Connors, K., Matheis, N., Schuster, D.K., Menasco, D.J., Wagoner, W., Lightner, J. and Wagner, D.R (2003). Activation Tagging in Tomato Identifies a Transcriptional Regulator of Anthocyanin Biosynthesis, Modification, and Transport. Plant Cell. 15: 1689–1703.

Matile, P. (2000). Biochemistry of Indian summer: physiology of autumnal leaf coloration. Experimental Gerontology. 35: 145-158.

Mattoo, A.K., Hoffman-Falk, H., Marder, J.B., Edelman, M. (1984). Regulation of proteinmetabolism: Coupling of photosynthetic electron transport to in vivo degradation of therapidly metabolized 32-kilodalton protein of the chloroplast membranes. Proceedings of theNational Academy of Sciences, USA. 81: 1380–1384.

Miras, S., Salvi, D., Ferro, M., Grunwald, D., Garin, J., Joyard, J., Rolland, N. (2002). Non-canonical transit peptide for import into the choloroplast. Journal of Biological Chemistry. 227: 4770-4778.

Moore S., Vrebalov J., Payton P, Giovannoni J. (2002). Use of genomics tools to isolate keyripening genes and analyse fruit maturation in tomato. Journal of Experimental Botany. 53:2023-2030.

Morstadt, L., Gräber, P., Pascalis, L., Kleinig, H., Speth, V., Beyer, P. (2002). Chemiosmotic ATP synthesis in photosynthetically inactive chromoplasts from Narcissus pseudonarcissus L. linked to a redox pathway potentially also involved in carotene desaturation. Planta. 215: 134-140.

Mulo, P., Pursiheimo, S., Hou, C.X., Tyystjarvi, T. and Aro, E.M. (2003). Multiple effects of antibiotics on chloroplast and nuclear gene expression. Functional Plant Biology.30: 1097-1103.

Mustilli, A.C., Fenzi, F., Ciliento, R., Alfano, F. and Bowler, C. (1999). Phenotype of the tomato high pigment-2 mutant is caused by a mutation in the tomato homolog of DEETIOLATED1. Plant Cell 11: 145-157.

Nada, M., Soll, J. (2004). Inner envelope protein 32 is imported into chloroplasts by a novel pathway. Journal of Journal of Cell Science ence. 117: 3975-3982.

Natesan, K. S. A., Sullivan, J.A., and Gray, J.C. (2005). Stromules: a characteristic cell-specific feature of plastid morphology. Journal of Experimetal Botany. 56: 787-797.

Neuhaus, H.E. and Emes, M.J. (2000). Non-photosynthetic metabolism in plastids. Annual Review of Plant Physiology and. Plant Molecular Biology. 51: 111-140.

Nomura T., Nakayama N., Murata T., and Akazawa T. (1967). Biosynthesis of Starch in Chloroplasts. Plant Physiology. 42: 327-332.

Nunan, K. J (1999). Cell wall metabolism in developing grape berries. PhD Thesis, The University of Adelaide, Australia.

Obiadalla-Ali, H., Fernie, A.R., Kossmann, J. and Lloyd, J.R. (2004). Developmental analysis of carbohydrate metabolism in tomato (Lycopersicon esculentum cv. Micro-Tom) fruits. Physiologia Plantarum.120: 196–204.

Obukosia, S.D., Richards, C.M. and Boyer, C.D. (2003). Expression of plastid-encoded photosynthetic genes during chloroplast or chromoplast differentiation in Cucurbitae pepo L. fruits. Phytochemistry. 64: 1213-1221.

Oeller, P.W., Lu, M.W., Taylor, L.P., Pike, D.A., Theologis, A. (1991). Reversible inhibition of tomato fruit senescence by antisense RNA. Science. 254:437–439.

Oke, M., Pinhero, R.G., Paliyath, G. (2003). The effects of genetic transformation of tomato with antisense phospholipase D cDNA on the quality characteristics of fruits and their processed products. Food Biotechnology. 17:163–182.

Ortiz-Serrano, P., Gil, J.V. (2007). Quantitation of free and glycosidically bound volatiles in and effect of glycosidase addition on three tomato varieties (Solanum lycopersicum L.). Journal of Agricultural and Food Chemistry. 55:9170–9176.

Ouyang, J., Shao, X., Li, J. (2000). Indole-3-glycerol phosphate, a branchpoint of indole-3-aceticacid biosynthesis from the tryptophan biosynthetic pathway in Arabidopsis thaliana. The Plant Journal. 24: 327-333.

Paolillo, D.J., Garvin, D.F. and Parthasarathy, M.V. (2004). The Chromoplasts of Or mutants of cauliflower (Brassica oleracea L. var. botrytis). Protoplasma. 224: 245-253.

Pecker, I., Chamovitz, D., Linden, H., Sandmann, G., Hirschberg, J. (1992). A single polypeptide catalyzing the conversion of phytoene to zeta-carotene is transcriptionally regulated during tomato fruit ripening. Proceeding of the National Academy of Sciences of the United States of America. 89:4962–4966.

Pecker, I., Gabbay, R., Cunningham Jr, F.X., Hirschberg, J. (1996). Cloning and characterization of the cDNA for lycopene beta-cyclase from tomato reveals decrease in its expression during fruit ripening. Plant Molecular Biology. 30: 807–819.

Peeters, N., Small, I. (2001). Dual targeting to mitochondria and chloroplasts. Biochimica et Biophysica Acta. 1541: 54-63.

Peltier, J.B., Cai, Y., Sun, O., Zabrouskov, V., Giacomelli, L., Rudella, A., Ytterberg, A.J., Rutschow, H. and Wijk K.J. (2006). The oligomeric stromal proteome of Arabidopsis thaliana chloroplasts. Molecular & Cell Proteomics. 5: 114-133.

Peltier; J.B., Emanuelsson, O., Kalume, D.E., Ytterberg, J., Friso, G., Rudella, A., Liberles, D.A., Soderberg, L., Roepstorff, P., von Heijne, G., van Wijk, K.J. (2002). Central functions of the luminal and peripheral thylakoid proteome of Arabidopsis determined by experimentation and genome-wide prediction. The Plant Cell. 14: 211-236.

Peltier, .J.B., Ytterberg, J., Sun, Q., van Wijk, K.J. (2004). New functions of the thylakoid membrane proteome of Arabidopsis thaliana revealed by a simple, fast, and versatile fractionation strategy. Journal of Biological Chemistry. 279:49367-49383.

Peltier, J.B., Cai, Y., Sun, O., Zabrouskov, V., Giacomelli, L., Rudella, A., Ytterberg, A.J., Rutschow, H., van Wijk, K.J. (2006). The oligomeric stromal proteome of Arabidopsis thaliana chloroplasts. Molecular & Cell Proteomics. 5, 114-133.

Piechulla B, Glick RE, Bahl H, Melis A, Gruissem W. (1987). Changes in photosyntheticcapacity and photosynthetic protein pattern during tomato fruit ripening. Plant Physiology. 84: 911-917.

Piechulla, B., Chonoles Imlay, K.R. and Gruissem, W. (1985). Plastid gene expression during fruit ripening in tomato. Plant Molecular Biology. 5: 373-385.

Pirrello, J., Regad, F., Latché, A., Pech, J.C. and Bouzayen, M. (2009). Regulation of Tomato Fruit Ripening. In Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. CAB Reviews. 4: 1-14.

Preberg, T., Wrisher, M., Fulgosi, H. and Ljubesic, N. (2008). Ultrastructural characterization of the reversible differentiation of chloroplasts in cucumber fruit. Journal of Plant Physiology.51: 122-131.

Pruzinska, A., Tanner, G., Aubry, S., Anders, I., Moser, S., Müller T., Ongania, K.H., Kräutler, B., Youn, J.Y., Liljegren, S.J., Hörtensteiner, S. (2005). Chlorophyll breakdown in senescentArabidopsis leaves. Characterization of chlorophyll catabolites and of chlorophyll catabolicenzymes involved in the degreening reaction. Plant Physiology. 139: 52-63.

Pyke, K.A. (2007). Plastid biogenesis and differentiation. In Topics in Current Genetics, Cell and molecular biology of plastids. Edited by Bock, R. 19:1-28. Springer-Verlag, Berlin, Heidelberg.

Pyke, K.A. and Howells, C.A. (2002). Plastid and stromule morphogenesis in tomato. Annals of Botany. 90: 559-566.

Railton, I.D., Fellows, B., West, C.A. (1984). Ent-Kaurene synthesis in chloroplasts from higherplants. Phytochemistry. 23: 1261-1267.

Rapp, J.C. and Mullet, J.E. (1991). Chloroplast transcription is required to express the nuclear genes rbcs and cab plastid DNA copy number is regulated independently. Plant Molecular Biology. 17: 813-823.

Rappsilber, J., Ryder, U., Lamond, A. I., and Mann, M. (2002). Large-scale proteomic analysis of the human spliceosome. Genome Research. 12: 1231–1245.

Ravanel, S., Block, M.A., Rippert, P., Jabrin, S., Curien, G., Rébeillé, F., Douce, R. (2004). Methionine metabolism in plants. Chloroplasts are autonomous for de novo methionine synthesis and can import S-adenosylmethionine from the cytosol. Journal of Biological Chemistry. 279: 22548-22557.

Ravi, V., Khurana, J.P., Tyagi, A.K. and Khurana, P. (2008). An update on chloroplast genomes. Plant systematics and evolution. 271: 101-122.

Ren, G., An, K., Liao, Y., Zhou, X., Cao, Y., Zhao, H., Ge, X., Kuai, B.(2007). Identification of a novel chloroplast protein AtNYE1 regulating chlorophyll degradation during leaf senescence in Arabidopsis. Plant Physiology. 144: 1429-1441.

Richards, C.M., Hinman, S.B., Boyer, C.D., Hardison, R.C. (1991). Survey of plastid RNA abundance during tomato fruit ripening: the amounts of RNA from the ORF2280 region increases in chromoplasts. Plant Molecular Biology. 17: 1179-1188.

Rodrigo, M.J. and Zacarias, L. (2007). Effect of postharvest ethylene treatment on carotenoid accumulation and the expression of carotenoid biosynthetic genes in the flavedo of orange (Citrus sinensis L. Osbeck) fruit. Postharvest Biology and Technology. 43: 14-22.

Rohmer, M. (1999). The discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants. Natural Product Report. 16:565–574.

Roje, S. (2007). Vitamin B biosynthesis in plants. Phytochemistry. 68: 1904-1921.

Rolland, F., Sheen, J. (2005). Sugar sensing and signalling networks in plants. Biochemical Society Transactions. 33: 269-271.

Rolland, N. (2003). Proteomics of the chloroplast enveloppe membranes from Arabidopsis thaliana. Molecular & Cell Proteomics. 2: 325-345.

Ronen, G., Cohen, M., Zamir, D., Hirschberg, J. (1999). Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon-cyclase is down-regulated during ripening and is elevated in the mutant Delta. The Plant Journal. 17:341–351.

Rose, J. K. C., Lee, H. H. and Bennett, A. B. (1997) Expression of a divergent expansin gene is fruit-specific and ripening-regulated. Proceedings of the National Academy of Sciences. USA 94. 5955–5960.

Rutschow, H., Ytterberg, A.J., Friso, G., Nilssonn R., van Wijk, K.J. (2008). Quantitative proteomics of a chloroplast SRP54 sorting mutant and its genetic interaction with CLPC1 in Arabidopsis. Plant Physiology. 148: 156-175.

Sadowski, P.G.; Dunkley, T.P.J.; Shadforth, I.P., Dupree P., Bessant C., Griffin J.L, Lilley K.S., (2006). Quantitative proteomic approach to study subcellular localization of membrane proteins. Nature Protocols. 1: 1778-1789.

Schelbert, S., Aubry, S., Burla, B., Agne, B., Kessler, F., Krupinska, K., Hörtensteiner, S. (2009). Pheophytin pheophorbide hydrolase (pheophytinase) is involved in chlorophyll breakdown during leaf senescence in Arabidopsis. The Plant Cell. 21: 767-785.

Schubert, M., Petersson, U.A., Haas, B.J., Funk, C., Schroder, W.P., Kieselbach, T. (2002). Proteome map of the chloroplast lumen of Arabidopsis thaliana. Journal of Biological Chemistry. 277: 8354-8365.

Schulz, A; Knoetzel, J; Scheller, HV, Mant, A. (2004). Uptake of a fluorescent dye as a swift and simple indicator of organelle intactness: Import-competent chloroplasts from soil-grown Arabidopsis. Journal of Histochemistry & Cytochemistry. 52: 701-704.

Schwender, J., Goffman, F., Ohlrogge, J.B., Shachar-Hill, Y. (2004). Rubisco without the Calvin cycle improves the carbon efficiency of developing green seeds. Nature. 432: 779-782.

Shahbazi, M., Gilbert, M., Labouré, A.M., Kuntz, M. (2007). Dual role of the plastid terminal oxidase in tomato. Plant Physiology. 145: 691-702.

Showalter, A.M. (1993). Structure and function of plant cell wall proteins. Plant Cell. 5: 9-23.

Siddique, M.A., Grossmann, J., Gruissem, W. and Baginsky, S. (2006) Proteome analysis of bell pepper (Capsicum annuum L.) chromoplasts. Plant Cell Physiol. 47: 1663-1673.

Simkin, A.J., Gaffé, J., Alacaraz, J.P., Carde, J.P., Bramley, P.M., Fraser, P.D., Kuntz, M. (2007). Fibrillin influence on plastid ultrastructure and pigment content in tomato fruit. Phytochemistry. 68: 1545-1556.

Simkin, A.J., Schwartz, S.H., Auldridge, M., Taylor, M.G., Klee, H.J. (2004). The tomato carotenoid cleavage dioxygenase 1 genes contribute to the formation of the flavor volatiles beta-ionone, pseudoionone, and geranylacetone. The Plant Journal. 40:882–892.

Sitte, P. (1987). Development and division of chromoplasts in petals of Forsythia. Cellule. 74: 59-77.

Škaloud, P., Neustupa, J., Radochova, B., Kubinova, L. (2005). Confocal microscopy of chloroplast morphology and ontogeny in three strains of Dictyochloropsis (Trebouxiophyceae, Chlorophyta). Phycologia. 44: 261–269.

Škaloud, P. and Radochová, B. (2004). Confocal microscopy of the green-algal chloroplast. Czech Phycology, Olomouc, 4: 183-190.

Soll; J.(2002). Protein import into chloroplasts. Current Opinion in Plant Biology. 5: 529-535.

Speirs, J., Lee, E., Holt, K., Yong-Duk, K., Steele, N., Loveys, B., Schuch, W. (1998). Genetic manipulation of alcohol dehydrogenase levels in ripening tomato fruit affects the balance of some flavor aldehydes and alcohols. Plant Physiology.117: 1047–1058.

Spurr, A.R. and Harris, W.M. (1968). Ultrastructure of chloroplasts and chromoplasts in Capsicum annuum. I. Thylakoid membrane changes during fruit ripening. American Journal of Botany. 55: 1210-1224.

Stange, C., Fuentes, P., Handford, M. and Pizarro, L. (2008). Daucus carota as a novel model to evaluate the effect of light on carotenogenic gene expression. Biological Research. 41: 289-301.

Steinhauser, M.C., Steinhauser, D., Koehl, K., Carrari, F., Gibon, Y., Fernie, A.R. and Stitt, M. (2010). Enzyme activity profiles during fruit development in tomato cultivars and Solanum pennellii. Plant Physiology.153: 80–98.

Stepanova, A.N., Hoyt, J.M., Hamilton, A.A., Alonso, J.M. (2005). A link between ethylene and auxinuncovered by the characterization of two root-specific ethylene-insensitive mutants inArabidopsis. The Plant Cell. 17: 2230-2242.

Strand, A., Asami, T., Alonso, J., Ecker, J.R., Chory, J. (2003). Chloroplast to nucleus communication triggered by accumulation of Mg-protoporphyrin IX. Nature. 421: 79-83.

Sugiura, M. (1992). The chloroplast genome. Plant Molecular Biology. 19: 149-168.

Sun, Q., Zybailov, B., Majeran, W., Friso, G., Olinares, P.D., van Wijk, K.J. (2008). PPDB, the Plant Proteomics Database at Cornell. Nucleic Acids Research. 37: D979-D974.

Surpin, M., Larkin, R.M., Chory, J. (2002). Signal transduction between the chloroplast and thenucleus. The Plant Cell. 14: S327-S338.

Tadmor, Y. (2002). Identification of malodorous, a wild species allele affecting tomato aroma that was selected against during domestication. Journal of Agriculture and Food Chemistry. 50:2005–2009.

Tanksley, S.D. and Nelson, J.C. (1996). Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theoretical and Applied Genetics. 92:191-203.

Terry, M.J., Lagarias, J.C. (1991). Holophytochrome assembly. Coupled assay forphytochromobilin synthase in organello. Journal of Biological Chemistry. 266: 22215-22221.

Tetlow, I.J., Bowsher, G.G., Emes, M.J. (2003). Biochemical properties and enzymatic capacities of chromoplasts isolated from wild buttercup (Ranunculus acris L.). Plant Science. 165: 383-394.

Thimm, O., Blasing, O., Gibon, Y., Nagel, A., Meyer, S., Kruger, P., Selbig, J., Muller, L.A., Rhee, S.Y., Stitt, M. (2004). MAPMAN: a user-driven tool to display genomics data sets onto diagrams ofmetabolic pathways and other biological processes. The The Plant Journal. 37: 914-939.

Thom, E., Möhlmann, T., Quick, W.P., Camara, B., Neuhaus, H.E. (1998). Sweet pepper plastids: enzymatic equipment, characterization of the plastidic oxidative pentose-phosphate pathway,and transport of phosphorylated intermediates across the envelope membrane. Planta. 204: 226-233.

Thomas, H., Huang, L., Young, M., Ougham, H. (2009). Evolution of plant senescence. BMC Evolutionary Biology 9, 163 doi:10.1186/1471-2148-9-163.

Thomson, W.W. and Whatley, J.M. (1980). Development of nongreen plastids. Annual Review of Plant Physiology and Plant Molecular Biology. 31: 375-394.

Thomson, W.W., Lewis, L.N. and Coggins, C.W. (1967). The reversion of chromoplasts to chloroplasts in Valencia oranges. Cytologia. 32: 117-124.

Tieman, D., Taylor, M., Schauer, N., Fernie, A.R., Hanson, A.D., Klee, H.J. (2006). Tomato aromatic amino acid decarboxylases participate in synthesis of the flavor volatiles 2-phenylethanol and 2-phenylacetaldehyde. Proceedings of the National Academy of Sciences of the United States of America.103:8287–8292.

Trainotti, L., Pavanello, A., Casadoro, G. (2005). Different ethylene receptors show an increased expression during the ripening of strawberries: does such an increment imply a role for ethylene in the ripening of these non-climacteric fruits? Journal of Experimental Botany. 56:2037–2046.

Uniacke, J. and Zerges, W. (2009). Chloroplast protein targeting involves localized translation in Chlamydomonas, Proceedings of the National Academy of Sciences. 106: 1439–1444

van Wijk, K.J., Peltier, J.B., Giacomelli, L. (2007). Isolation of chloroplast proteins from Arabidopsis thaliana for proteome analysis. Methods in Molecular Biology. 355: 43-48.

Vasquez-Caicedo, A.L., Heller, A., Neidhart, S. and Carle, R. (2006). Chromoplast morphology and β-carotene accumulation during postharvest ripening of Mango Cv. “Tommy Atkins”. Journal of Agricultural and Food Chemistry. 54: 5769-5776.

Vidi, P.A., Kanwischer, M., Baginski, S., Austin, J.A., Csucs, G., Dörmann, P., Kessler, F., Bréhélin, C. (2006). Tocopherol cyclase (VTE1) localization and vitamin E accumulation in chloroplastsplastoglobule lipoprotein particles. Journal of Biological Chemistry. 281: 11225-11234.

von Zychlinski, A. , Kleffmann, T., Krishnamurthy, N., Sjölander, K., Baginsky, S., Gruissem, W. (2005). Proteome analysis of the rice etioplast: metabolic and regulatory networks and novel protein functions. Molecular & Cell Proteomics. 4: 1072-1084

Vothknecht, U.C., Soll, J. (2005). Chloroplast membrane transport: interplay of procaryotic and eucaryotic traits. Gene. 354: 99-109.

Vrebalov, J., Ruezinsky, D., Padmanabhan, V., White, R., Medrano, D., Drake, R., Schuch, W. and Giovannoni, J. (2002). A MADS-box gene necessary for fruit ripening at the tomato Ripening-inhibitor (Rin) locus. Science. 296: 343–346.

Wada, S., Ishida, H., Izumi, M., Yoshimoto, K., Ohsumi, Y., Mae, T., Makino, A. (2009). Autophagyplays a role in chloroplast degradation during senescence in individually darkened leaves. Plant Physiology. 149: 885-893.

Walker, D.A:, Cerovic, Z.G., Robinson, S. (1987). Isolation of intact chloroplasts: general principles and criteria of integrity. Methods Enzymol. 148: 1445-157.

Wang, S., Liu, J., Feng, Y., Niu, X., Giovannoni, J. and Liu, Y. (2008). Altered plastid levels and potential for improved fruit nutrient content by down-regulation of the tomato DDB1-interacting protein CUL4. The Plant Journal. 55: 89–103.

Waters, M.T. and Pyke, K.A. (2004). Plastid development and differentiation. In Plastids: Edited by Møller, S.G., pp. 30-59. Blackwell, Oxford, UK.

Waters, M.T., Fray, R.G. and Pyke, K.A. (2004). Stromule formation is dependent upon plastid size, plastid differentiation status and the density of plastids within the cell. The The Plant Journal. 39: 655-667.

Westphal, S., Soll, J. and Vothknecht, U.C. (2001). A vesicle transport system inside chloroplasts. FEBS Letters. 506: 257–261.

Whatley, J.M., McLean, B., Juniper, B.E. (1991). Continuity of chloroplast and endoplasmic reticulum membranes in Phaseolus vulgaris. New Phytologist. 117: 209-217.

Whatley, J.M. (1978). A suggested cycle of plastid development interrelationships. New Phytologist. 80: 489-502.

Wiese, A., Gröner, F., Sonnewald, U., Deppner, H., Lerchl, J., Hebbeker, U., Flügge, U.I., Weber, A. (1999). Spinach hexokinase I is located in the outer envelope membrane of plastids. FEBS Letters. 461: 13-18.

Wilkinson, J.Q., Lanahan, M.B., Yen, H.C., Giovannoni, J.J. and Klee, H.J. (1995). An ethylene-inducible component of signal-transduction encoded by NEVER-RIPE. Science. 270: 1807-1809.

Wilm, M., Shevchenko, A., Houthaeve, T., Breit, S., Schweigerer, L., Fotsis, T., Mann, M.(1996). Femtomole sequencing of proteins from polyacrylamide gels by nano-electrospray mass spectrometry. Nature. 379: 466-469.

Yamaguchi, K., Subramanian, A.R. (2003). Proteomic identification of all plastid-specificribosomal proteins in higher plant chloroplast 30S ribosomal subunit PSRP-2 (U1A-typedomains), PSRP-3a/b (ycf65 homologue) and PSRP-4 (Thx homologue). European Journal of Biochemistry. 270: 190–205.

Yang, S.F., Hoffman, N.E. (1984). Ethylene biosynthesis and its regulation in higher plants. Annual Review Plant Physiology. 35:155–189.

Yen, H., Shelton, A., Howard, L., Vrebalov, J. and Giovannoni, J.J. (1997). The tomato high pigment (hp) locus maps to chromosome 2 and influences plastome copy number and fruit quality. Theoretical and Applied Genetics. 95: 1069-1079.

Yoo, S.D., Cho, Y.H., Tena, G., Xiong, Y., Sheen J. (2008). Dual control of nuclear EIN3 by bifurcate MAPK cascades in C2H4 signalling. Nature. 451:789–795.

Ytterberg, A.J., Peltier, J.B. and van Wijk, K.J. (2006). Protein profiling of plastoglobules in chloroplasts and chromoplasts. A surprising site for differential accumulation of metabolic enzymes. Plant Physiology.140: 984-997.

Yu, T.S., Kofler, H., Häuser, R.E., Hille, D., Flügge, U.I., Zeeman, S.C., Smith, A.M., Kossmann, J., Lloyd, J., Ritte, G., Steup, M., Lue, W.L., Chen, J., Weber, A. (2001). The Arabidopsis sex1 mutant is defective in the R1 protein, a general regulator of starch degradation in plants, andnot in the chloroplast hexose transporter. The Plant Cell. 13: 1907-1918.

Zhang, M., Yuan, B. and Leng, P. (2009). The role of ABA in triggering ethylene biosynthesis and ripening of tomato fruit. Journal of Experimetal Botany. 60: 1579-1588.

Zhou, X., Van Eck, J. and Li, L. (2008). Use of the cauliflower Or gene for improving crop nutritional quality, Biotechnology Annual Review. 14: 171-190.

Zybailov, B., Friso, G., Kim, J., Rudella, A., Ramirez Rodriguez, V., Asakura, Y., Sun, Q., van Wijk, K.J. (2009). Large scale comparative proteomics of a chloroplast Clp protease mutantreveals folding stress, altered protein homeostasis and feedback regulation of metabolism. Molecular & Cell Proteomics. 8, 1789-1810.

Zybailov, B., Rutschow, H., Friso, G., Rudella, A., Emanuelsson, O., Sun, Q., van Wijk, K.J. (2008). Sorting signals, N-terminal modifications and abundance of the chloroplast proteome. PLoS One 3, e1994.

Articles related to the subject

Sanchez-Bel P., Barsan C., Rombaldi C., Egea A., Latché A., Pech J.C. Molecular biology and biotechnology for fresh fruit quality: the role of chromoplast. Acta Hort. 837: 339-344. (2009).

Barsan C, Paloma Sanchez-Bel P., Rombaldi C., Rossignol M., Kuntz M., Zouine M., Latché A., Bouzayen M. et Pech J.C. Metabolic and regulatory features of the tomato chromoplast revealed by proteomic analysis **,** J.Ex.Bot 61(9):2413-2431. (2010)

Barsan C., Egea I., Bian W., Purgatto E., Latché A., Chervin C., Bouzayen M. and Pech J.C *Chromoplast differentiation: current status and future prospect.* Accepted with minor modifications in Plant cell Physiology. (2010

Barsan C., Egea I., Bian W., Purgatto E., Latché A, Chervin C., Bouzayen M. and Pech J.C Metabolic and molecular events occurring during the biogenesis of chromoplasts- Submitted to Journal of Botany, September 2010

Oral communications

Pech J.C., Barsan C., Sanchez-Bel P., Egea I., Rombaldi C., Latché A. 2008. Biología molecular y biotecnología de la maduración de frutos. Proceedings of the IX National and VI Iberic Symposium on "Maduracion y postcosecha" 23-26 september 2008, Zaragoza, Spain, Dr Rosa Oria, Ed, 7 p

Posters

Sanchez-Bel P., Barsan C., Zouine M., Diouri F., Borderies G., Pichereaux C., Latché A., Rossignol M., Pech J.C., (2007) Analyse protéomique de la transition chloroplaste- chromoplaste au cours de la maturation de la tomate. Colloque Spectrométrie de Masse et Analyse Protéomique. Pau, France. Poster.

Barsan C., Sanchez-Bel P., Rombaldi C., Rossignol M., Kuntz M., Zouine M., Latché A., Bouzayen M., and Pech JC. 2009. Extensive analysis of the tomato chromoplast proteome reveals specific

metabolic and regulatory features. Congrès français de Spectrométrie de Masse et d'Analyse Protéomique (SMAP 2009), Dijon, France. Poster.

Cristina Barsan, Isabel Egea, Paloma Sanchez-Bel, Eduardo Purgatto, Carole Pichereaux, Mohamed Zouine, Alain Latche, Mondher Bouzayen, Jean-Claude Pech , Michel Rossignol Analysis of the tomato chromoplast proteome reveals specific metabolic and regulatory features (SMAP 2010), Marseille, France. Poster

ANNEX

SGN	AT	mapman code	mapman class
SGN-U312593	AT3G47470	1.1.2.01	PS.lightreaction.photosystem I.LHC-I
SGN-U317042	AT3G47470	1.1.2.01	PS.lightreaction.photosystem I.LHC-I
SGN-U315267	AT2G40490	19.07	tetrapyrrole synt.uroporphyrinogen decarboxylase
SGN-U318908	AT5G63890	13.1.7.08	aa metab.synt.histidine.histidinol dehydrogenase
SGN-U315064	AT5G13420	7.2.02	OPP.non-reductive PP.transaldolase
SGN-U314201	AT3G17240	8.1.01.03	TCA / org. transformation.TCA.pyruvate DH.E3
SGN-U312874	AT1G20696	27.3.62	RNA.regulation of transcription.
SGN-U320344	AT1G15690	34.30	transport.H+ transporting pyrophosphatase
SGN-U320084	AT2G47390	35.2	not assigned.unknown
SGN-U321526	AT4G16170	35.2	not assigned.unknown
SGN-U319267	AT5G58250	35.2	not assigned.unknown
SGN-U343103	AT5G12290	35.1	not assigned.no ontology
SGN-U321522	AT1G34430	8.1.01.02	TCA / org. transformation.TCA.pyruvate DH.E2
SGN-U316982	AT5G53480	29.3.1	protein.targeting.nucleus
SGN-U314162	AT2G34590	11.1.031	lipid metab.FA synt. and FA elongation.pyruvate DH
SGN-U312668	AT1G69740	19.04	tetrapyrrole synt.ALA dehydratase
SGN-U319107	AT1G22700	35.1.5	not assigned.
SGN-U313426	AT1G15690	34.30	transport.H+ transporting pyrophosphatase
SGN-U312661	AT5G01530	1.1.1.01	PS.lightreaction.photosystem II.LHC-II
SGN-U312611	AT2G05710	8.1.03	TCA / org. transformation.TCA.aconitase
SGN-U315733	AT2G31670	35.1	not assigned.no ontology
SGN-U324760	AT1G77090	1.1.1.2	PS.lightreaction.photosystem II.PSII polypeptide subunits
SGN-U329685	AT5G16930	29.5.09	protein.degrad.AAA type
SGN-U323134	AT3G25660	19.01	tetrapyrrole synt.glu-tRNA synthetase
SGN-U319463	AT1G15390	26.01	misc.misc2
SGN-U317466	AT2G45300	13.1.6.1.06	aa metab.synt.aromatic aa.chorismate
SGN-U313975	AT4G24280	20.2.1	stress.abiotic.heat
SGN-U315717	AT5G49910	20.2.1	stress.abiotic.heat
SGN-U335973	AT2G38550	35.2	not assigned.unknown
SGN-U314203	AT3G61440	13.1.5.3.01	aa metab.synt.serine-glycine-cysteine group.cysteine.OASTL
SGN-U314288	AT1G74310	20.2.1	stress.abiotic.heat
SGN-U312436	AT2G05100	1.1.1.01	PS.lightreaction.photosystem II.LHC-II
SGN-U313211	AT1G29930	1.1.1.01	PS.lightreaction.photosystem II.LHC-II
SGN-U313212	AT1G29930	1.1.1.01	PS.lightreaction.photosystem II.LHC-II
SGN-U313213	AT1G29930	1.1.1.01	PS.lightreaction.photosystem II.LHC-II
SGN-U313204	AT1G29930	1.1.1.01	PS.lightreaction.photosystem II.LHC-II
SGN-U312339	AT1G15820	1.1.1.01	PS.lightreaction.photosystem II.LHC-II
SGN-U331670	ATCG00720	1.1.03	PS.lightreaction.cytochrome b6/f
SGN-U317741	AT2G38040	16.99	secondary metab.unspecified
SGN-U312538	AT2G28000	1.3.013	PS.calvin cyle.rubisco interacting

SGN-U314204	AT3G61440	13.1.5.3.01	aa metab.synt.serine-glycine-cysteine group.cysteine.OASTL
SGN-U316879	AT1G11430	33.99	development.unspecified
SGN-U315305	AT1G59900	8.1.01.01	TCA / org. transformation.TCA.pyruvate DH.E1
SGN-U316102	AT2G30200	11.1.02	lipid metab.FA synt. and FA elongation.Acetyl CoA Transacylase
SGN-U318835	AT3G15730	11.9.3.01	lipid metab.lipid degrad.lysophospholipases.phospholipase D
SGN-U312461	AT3G26650	1.3.04	PS.calvin cyle.GAP
SGN-U315784	AT4G14880	13.1.5.3.01	aa metab.synt.serine-glycine-cysteine group.cysteine.OASTL
SGN-U316606	AT3G59760	13.1.5.3.01	aa metab.synt.serine-glycine-cysteine group.cysteine.OASTL
SGN-U315993	AT1G03475	19.08	tetrapyrrole synt.coproporphyrinogen III oxidase
SGN-U316629	AT1G56500	35.1	not assigned.no ontology
SGN-U312377	AT4G30920	29.5	protein.degrad.
SGN-U312543	AT2G39730	1.3.013	PS.calvin cyle.rubisco interacting
SGN-U321504	AT2G29690	13.1.6.5.01	aa metab.synt.aromatic aa.tryptophan.anthranilate synth.
SGN-U321505	AT2G29690	13.1.6.5.01	aa metab.synt.aromatic aa.tryptophan.anthranilate synth.
SGN-U316417	AT3G29320	2.2.2.02	major CHO metab.degrad.starch.starch phosphorylase
SGN-U312640	AT1G03130	1.1.2.2	PS.lightreaction.photosystem I.PSI polypeptide subunits
SGN-U312531	AT3G50820	1.1.1.2	PS.lightreaction.photosystem II.PSII polypeptide subunits
SGN-U312863	AT2G17390	27.3.39	RNA.regulation of transcription.
SGN-U318405	AT5G28540	20.2.1	stress.abiotic.heat
SGN-U324190	AT2G43710	11.1.015	lipid metab.FA synt. and FA elongation.ACP desaturase
SGN-U315884	AT4G15440	35.1	not assigned.no ontology
SGN-U313729	AT2G21170	1.3.05	PS.calvin cycle.TPIf
SGN-U315883	AT3G05970	11.1.09	lipid metab.FA synt. and FA elongation.
SGN-U313474	AT3G55290	26.22	misc.short chain dehydrogenase/reductase (SDR)
SGN-U312574	AT3G10050	13.1.4.5.01	aa metab.synt.branched chain group.isoleucine specific.t
SGN-U315069	AT3G02780	16.1.2.07	secondary metab.isoprenoids.mevalonate pathway.
SGN-U314967	AT2G02010	13.1.1.1.01	aa metab.synt.central aa metab.GABA.
SGN-U312830	AT3G03250	4.01	glycolysis.UGPase
SGN-U313381	AT3G63410	16.1.3.03	secondary metab.isoprenoids.tocopherol biosynt.
SGN-U315586	AT1G10070	13.1.4.1.04	aa metab.synt.branched chain group.
SGN-U316590	AT1G12250	35.1	not assigned.no ontology
SGN-U315101	AT3G16240	34.19.2	transport.Major Intrinsic Proteins.TIP
SGN-U316550	AT3G25780	17.7.1.04	hormone metab.jasmonate.synt.-degrad.allene oxidase cyclase
SGN-U319065	AT4G04910	29.5.09	protein.degrad.AAA type
SGN-U316542	AT3G23940	35.1	not assigned.no ontology
SGN-U316509	AT1G62750	29.2.4	protein.synt.elongation
SGN-U324505	AT3G52750	31.2	cell.division
SGN-U328370	AT2G36250	31.2	cell.division
SGN-U316984	AT2G39800	13.1.2.2.01	aa metab.synt.glutamate family.
SGN-U317732	AT3G52180	2.2.2	major CHO metab.degrad.starch
SGN-U325822	AT1G06950	29.3.3	protein.targeting.chloroplast
SGN-U314000	AT1G32790	27.4	RNA.RNA binding
SGN-U320414	AT1G71480	29.3.1	protein.targeting.nucleus
SGN-U320415	AT1G71480	29.3.1	protein.targeting.nucleus

SGN-U317424	AT1G51560	35.2	not assigned.unknown
SGN-U323701	AT1G34430	8.1.01.02	TCA / org. transformation.TCA.pyruvate DH.E2
SGN-U321169	AT1G52560	20.2.1	stress.abiotic.heat
SGN-U316408	AT1G79440	8.2.99	TCA / org. transformation.
SGN-U313926	AT1G22450	9.07	mitochondrial electron transport
SGN-U316689	AT1G47260	9.1.1.05	mitochondrial electron transport /
SGN-U320103	AT1G67280	24.02	Biodegrad. of Xenobiotics.lactoylglutathione lyase
SGN-U321330	AT1G67280	24.02	Biodegrad. of Xenobiotics.lactoylglutathione lyase
SGN-U322218	AT1G61790	34.3	transport.aas
SGN-U332979	AT1G17870	29.5.07	protein.degrad.metalloprotease
SGN-U316358	AT1G74030	4.012	glycolysis.enolase
SGN-U329529	AT1G73980	23.3.2.02	nucleotide metab.salvage.nucleoside kinases.uridine kinase
SGN-U318089	AT1G75330	13.1.2.3.021	aa metab.synt.glutamate family.arginine.
SGN-U337344	AT1G75330	13.1.2.3.021	aa metab.synt.glutamate family.arginine.
SGN-U323149	AT1G75420	35.1	not assigned.no ontology
SGN-U330695	AT1G25220	13.1.6.5.01	aa metab.synt.aromatic aa.tryptophan.anthranilate synth.
SGN-U313753	AT1G01090	11.1.031	lipid metab.FA synt. and FA elongation.pyruvate DH
SGN-U320340	AT1G14810	13.1.3.6.1.02	aa metab.synt.aspartate family.misc.homoserine.
SGN-U316862	AT2G43090	35.1.23	not assigned.
SGN-U317764	AT2G39020	26.24	misc.GCN5-related N-acetyltransferase
SGN-U315837	AT2G32520	26.01	misc.misc2
SGN-U332030	AT2G26730	30.2.3	signalling.receptor kinases.leucine rich repeat III
SGN-U312631	AT2G33150	13.2.4.1	aa metab.degrad.branched-chain group.shared
SGN-U321027	AT2G33740	15.2	metal handling.binding, chelation and storage
SGN-U314788	AT2G01140	1.3.06	PS.calvin cyle.aldolase
SGN-U312542	AT2G28000	1.3.013	PS.calvin cyle.rubisco interacting
SGN-U319649	AT2G01720	29.7	protein.glycosylation
SGN-U322488	AT2G16660	33.99	development.unspecified
SGN-U317686	AT2G23070	29.4	protein.postranslational modification
SGN-U343019	AT2G17980	29.3.4.4	protein.targeting.secretory pathway.plasma membrane
SGN-U321518	AT3G10370	11.5.03	lipid metab.glyceral metab.
SGN-U319639	AT3G48680	9.1.1.05	mitochondrial electron transport /
SGN-U315956	AT3G61870	35.2	not assigned.unknown
SGN-U316138	AT3G56460	26.01	misc.misc2
SGN-U313625	AT3G63490	29.2.1.1.1.2.01	protein.synt.ribosomal protein.prokaryotic
SGN-U316540	AT3G23940	35.1	not assigned.no ontology
SGN-U316069	AT3G18490	27.3.99	RNA.regulation of transcription.unclassified
SGN-U319875	AT3G11830	29.6	protein.folding
SGN-U312793	AT3G58610	13.1.4.1.02	aa metab.synt.branched chain group.common
SGN-U312795	AT3G58610	13.1.4.1.02	aa metab.synt.branched chain group.common
SGN-U318568	AT3G45740	35.1	not assigned.no ontology
SGN-U316106	AT3G53580	13.1.3.5.04	aa metab.synt.aspartate family.lysine.
SGN-U321587	AT3G08640	35.1	not assigned.no ontology
SGN-U316338	AT3G60210	29.6	protein.folding

SGN-U317036	AT3G20820	35.1	not assigned.no ontology
SGN-U316718	AT3G15190	29.2.1.1.1.1.020	protein.synt.ribosomal protein..30S subunit.S20
SGN-U318067	AT3G15360	21.01	redox.thioredoxin
SGN-U316483	AT3G46740	29.3.3	protein.targeting.chloroplast
SGN-U319751	AT1G52670	35.1	not assigned.no ontology
SGN-U324780	AT3G09580	16.1.4	secondary metab.isoprenoids.carotenoids
SGN-U317040	AT3G55330	1.1.1.2	PS.lightreaction.photosystem II.PSII polypeptide subunits
SGN-U319086	AT4G35860	30.5	signalling.G-proteins
SGN-U317080	AT4G21210	35.2	not assigned.unknown
SGN-U316915	AT4G38460	16.1.1.010	secondary metab.isoprenoids.non-mevalonate pathway
SGN-U312673	AT4G38510	34.1.01	transport.p- and v-ATPases.H+-transporting two-sector ATPase
SGN-U315386	AT4G36910	35.1	not assigned.no ontology
SGN-U317882	AT4G36910	35.1	not assigned.no ontology
SGN-U320843	AT4G36910	35.1	not assigned.no ontology
SGN-U321665	AT4G20070	29.5	protein.degrad.
SGN-U321621	AT4G28390	34.9	transport.
SGN-U327379	AT4G34200	13.1.5.1.01	aa metab.
SGN-U317629	AT4G17300	29.1.022	protein.aa activation.asparagine-tRNA ligase
SGN-U316216	AT4G26300	29.1.019	protein.aa activation.arginine-tRNA ligase
SGN-U319357	AT4G10750	35.1	not assigned.no ontology
SGN-U326288	AT4G26900	13.1.7.010	aa metab.synt.histidine.Imidazole glycerol phosphate synth.
SGN-U313358	AT4G11010	23.4.010	nucleotide metab.
SGN-U320976	AT5G58270	34.16	transport.ABC transporters and multidrug resistance systems
SGN-U322433	AT5G52520	29.1.040	protein.aa activation.bifunctional aminoacyl-tRNA synthetase
SGN-U312582	AT5G52920	11.1.030	lipid metab.FA synt. and FA elongation.pyruvate kinase
SGN-U317659	AT5G63980	23.2	nucleotide metab.degrad.
SGN-U337369	AT5G64270	27.1.1	RNA.processing.splicing
SGN-U320082	AT5G23890	35.1	not assigned.no ontology
SGN-U319205	AT5G10160	11.1.05	lipid metab.FA synt. and FA elongation.
SGN-U317735	AT5G42150	35.2	not assigned.unknown
SGN-U316983	AT5G53480	29.3.1	protein.targeting.nucleus
SGN-U315044	AT5G47840	23.4.01	nucleotide metab.phosphotransfer and pyrophosphatases.
SGN-U319142	AT5G47870	35.2	not assigned.unknown
SGN-U319707	AT5G47860	35.2	not assigned.unknown
SGN-U318203	AT5G48220	13.1.6.5.04	aa metab.synt.aromatic aa.
SGN-U314132	AT5G11520	13.1.1.2.01	aa metab.synt.central aa metab.
SGN-U318527	AT5G11490	31.4	cell.vesicle transport
SGN-U344386	AT5G11490	31.4	cell.vesicle transport
SGN-U332327	AT5G25320	13	aa metab.
SGN-U317088	AT5G19320	30.5	signalling.G-proteins
SGN-U316197	AT5G66530	3.5	minor CHO metab.others
SGN-U321456	AT5G37360	35.2	not assigned.unknown
SGN-U317245	AT5G38530	13.1.6.5.05	aa metab.synt.aromatic aa.tryptophan.tryptophan synth.
SGN-U319777	AT5G01500	34.9	transport.

SGN-U315247	AT5G01650	35.1	not assigned.no ontology
SGN-U323959	AT5G19820	35.1	not assigned.no ontology
SGN-U327066	AT4G16660	20.2.1	stress.abiotic.heat
SGN-U315742	AT1G12230	7.2.02	OPP.non-reductive PP.transaldolase
SGN-U330266	AT1G73100	27.3.69	RNA.regulation of transcription.
SGN-U314115	AT1G02560	29.5.5	protein.degrad.serine protease
SGN-U317966	AT2G44350	8.1.02	TCA / org. transformation.TCA.CS
SGN-U316173	AT3G15360	21.01	redox.thioredoxin
SGN-U315947	AT5G62790	16.1.1.02	secondary metab.isoprenoids.non-mevalonate pathway.DXR
SGN-U324924	AT5G61510	26.07	misc.oxidases - copper, flavone etc.
SGN-U312423	AT2G36390	2.1.2.03	major CHO metab.synt.starch.starch branching
SGN-U312427	AT2G36390	2.1.2.03	major CHO metab.synt.starch.starch branching
SGN-U315633	AT3G45140	17.7.1.02	hormone metab.jasmonate.synt.-degrad.lipoxygenase
SGN-U321151	AT1G17420	17.7.1.02	hormone metab.jasmonate.synt.-degrad.lipoxygenase
SGN-U338544	AT5G56600	31.1	cell.organisation
SGN-U312319	AT3G60750	1.3.08, 7.2.01	PS.calvin cyle.transketolase, OPP.non-reductive PP.
SGN-U313134	AT3G44880	33.99	development.unspecified
SGN-U312558	AT3G12500	20.1	stress.biotic
SGN-U314048	AT4G35000	21.2.1	redox.ascorbate and glutathione.ascorbate
SGN-U312967	AT1G44575	1.1.1.2	PS.lightreaction.photosystem II.PSII polypeptide subunits
SGN-U315580	AT4G26630	35.2	not assigned.unknown
SGN-U314489	AT3G18080	26.03	misc.gluco-, galacto- and mannosidases
SGN-U313568	AT2G37220	27.4	RNA.RNA binding
SGN-U314388	AT1G79920	20.2.1	stress.abiotic.heat
SGN-U314389	AT1G79920	20.2.1	stress.abiotic.heat
SGN-U314922	AT3G13470	29.6	protein.folding
SGN-U316742	AT1G55490	1.3.013	PS.calvin cyle.rubisco interacting
SGN-U321035	AT5G67030	17.1.1.1.01	hormone metab.abscisic acid.synt.-degrad.synt.
SGN-U312460	AT5G33320	34.8	transport.metabolite transporters at the envelope membrane
SGN-U317866	AT1G27680	2.1.2.01	major CHO metab.synt.starch.AGPase
SGN-U318293	AT5G24300	2.1.2.02	major CHO metab.synt.starch.starch synth.
SGN-U317294	AT1G80560	13.1.4.4.03	aa metab.synt.branched chain group.leucine specific.
SGN-U316056	AT5G30510	29.2.1.1.3.1.01	protein.synt.ribosomal protein.30S subunit.S1
SGN-U335457	AT5G30510	29.2.1.1.3.1.01	protein.synt.ribosomal protein.30S subunit.S2
SGN-U313451	AT5G14040	34.9	transport.
SGN-U319106	AT1G03600	1.1.1.2	PS.lightreaction.photosystem II.PSII polypeptide subunits
SGN-U313507	AT1G06690	3.5	minor CHO metab.others
SGN-U320380	AT1G10510	33.99	development.unspecified
SGN-U320971	AT1G10510	33.99	development.unspecified
SGN-U316232	AT1G10830	34.14	transport.unspecified cations
SGN-U314759	AT1G12410	29.5.5	protein.degrad.serine protease
SGN-U314760	AT1G12410	29.5.5	protein.degrad.serine protease
SGN-U316274	AT1G15140	35.1	not assigned.no ontology
SGN-U312633	AT1G16880	35.1	not assigned.no ontology

SGN-U314094	AT1G16890	29.5.11.03	protein.degrad.ubiquitin.E2
SGN-U326460	AT1G24610	35.1.13	not assigned.SET domain-containing protein
SGN-U318885	AT3G04550	35.2	not assigned.unknown
SGN-U319530	AT1G29700	35.2	not assigned.unknown
SGN-U323609	AT1G30360	20.2.3	stress.abiotic.drought/salt
SGN-U315787	AT2G15290	35.2	not assigned.unknown
SGN-U318166	AT1G31190	3.4.05	minor CHO metab.myo-inositol.inositol phosphatase
SGN-U327879	AT3G09250	35.2	not assigned.unknown
SGN-U312753	AT1G34470	34.10	transport.nucleotides
SGN-U319658	AT2G22360	20.2.1	stress.abiotic.heat
SGN-U321353	AT1G36320	35.2	not assigned.unknown
SGN-U347119	AT2G25140	20.2.1	stress.abiotic.heat
SGN-U314695	AT1G42960	35.2	not assigned.unknown
SGN-U318063	AT1G42960	35.2	not assigned.unknown
SGN-U316126	AT3G15660	21.04	redox.glutaredoxins
SGN-U319508	AT2G26340	35.2	not assigned.unknown
SGN-U319399	AT3G16950	8.1.01.03	TCA / org. transformation.TCA.pyruvate DH.E3
SGN-U346363	AT3G16950	8.1.01.03	TCA / org. transformation.TCA.pyruvate DH.E3
SGN-U329012	AT1G44790	35.1	not assigned.no ontology
SGN-U316386	AT2G27680	3.5	minor CHO metab.others
SGN-U321161	AT3G17465	29.2.1.1.1.2.03	protein.synt.ribosomal protein.chloroplast.50S subunit.L3
SGN-U319434	AT3G18420	35.1.5	not assigned.
SGN-U314651	AT3G18480	35.1	not assigned.no ontology
SGN-U321141	AT3G20320	35.1	not assigned.no ontology
SGN-U312730	AT1G49970	29.5.5	protein.degrad.serine protease
SGN-U318299	AT2G33845	35.1	not assigned.no ontology
SGN-U315973	AT1G51440	11.9.2.01	lipid metab.lipid degrad.lipases.triacylglycerol lipase
SGN-U318516	AT1G52220	35.2	not assigned.unknown
SGN-U315340	AT2G36290	35.1	not assigned.no ontology
SGN-U315342	AT2G36290	35.1	not assigned.no ontology
SGN-U315343	AT2G36290	35.1	not assigned.no ontology
SGN-U320355	AT2G37400	35.1	not assigned.no ontology
SGN-U318707	AT2G37660	35.2	not assigned.unknown
SGN-U312749	AT1G54780	35.1	not assigned.no ontology
SGN-U316117	AT2G39795	35.1	not assigned.no ontology
SGN-U316609	AT1G55160	35.2	not assigned.unknown
SGN-U313400	AT1G55190	35.1	not assigned.no ontology
SGN-U334323	AT1G55190	35.1	not assigned.no ontology
SGN-U322113	AT2G40060	35.2	not assigned.unknown
SGN-U318827	AT1G55480	35.2	not assigned.unknown
SGN-U321797	AT2G41490	29.7	protein.glycosylation
SGN-U324098	AT2G41680	21.01	redox.thioredoxin
SGN-U313030	AT2G44870	35.2	not assigned.unknown
SGN-U319100	AT3G32930	35.2	not assigned.unknown

SGN-U328395	AT2G47840	29.3.3	protein.targeting.chloroplast
SGN-U346935	AT2G47940	29.5.5	protein.degrad.serine protease
SGN-U326644	AT3G44340	29.3.4.2	protein.targeting.secretory pathway.golgi
SGN-U317521	AT1G65260	35.1	not assigned.no ontology
SGN-U315248	AT1G66670	29.5.5	protein.degrad.serine protease
SGN-U326502	AT3G49720	35.2	not assigned.unknown
SGN-U326232	AT1G69830	2.2.2.1	major CHO metab.degrad.starch.starch cleavage
SGN-U319393	AT3G52190	33.99	development.unspecified
SGN-U318304	AT3G52230	35.2	not assigned.unknown
SGN-U321976	AT1G70570	13.1.6.5.02	aa metab.synt.aromatic aa.tryptophan.
SGN-U313639	AT3G52990	4.013	glycolysis.PK
SGN-U314516	AT3G56490	29.4	protein.postranslational modification
SGN-U320403	AT1G74070	31.3.01	cell.cycle.peptidylprolyl isomerase
SGN-U319724	AT1G74640	35.2	not assigned.unknown
SGN-U316549	AT3G60190	26.17	misc.dynamin
SGN-U318347	AT4G01050	35.1.41	not assigned.hydroxyproline rich proteins
SGN-U323580	AT4G15510	1.1.1.2	PS.lightreaction.photosystem II.PSII polypeptide subunits
SGN-U314138	AT4G17040	29.5.5	protein.degrad.serine protease
SGN-U326001	AT5G05200	35.2	not assigned.unknown
SGN-U326860	AT5G05200	35.2	not assigned.unknown
SGN-U323967	AT4G17420	35.2	not assigned.unknown
SGN-U322111	AT5G05480	35.2	not assigned.unknown
SGN-U317892	AT5G08540	35.2	not assigned.unknown
SGN-U322269	AT4G25370	29.3.99	protein.targeting.unknown
SGN-U317707	AT4G26860	35.1	not assigned.no ontology
SGN-U321529	AT5G12470	35.2	not assigned.unknown
SGN-U321170	AT4G29060	29.2.4	protein.synt.elongation
SGN-U319157	AT5G14910	35.1	not assigned.no ontology
SGN-U319359	AT4G32520	1.2.05, 25.01	PS.photorespiration.
SGN-U319360	AT4G32520	1.2.05, 25.01	PS.photorespiration.
SGN-U316787	AT5G17170	29.8	protein assembly and cofactor ligation
SGN-U317153	AT4G33520	34.12	transport.metal
SGN-U333215	AT4G34090	35.2	not assigned.unknown
SGN-U336316	AT4G34120	35.1	not assigned.no ontology
SGN-U318118	AT5G17710	29.6	protein.folding
SGN-U316769	AT5G19620	35.1	not assigned.no ontology
SGN-U323777	AT4G39460	34.9	transport.
SGN-U332469	AT5G23140	29.5.5	protein.degrad.serine protease
SGN-U318082	AT5G49940	29.8	protein assembly and cofactor ligation
SGN-U317645	AT5G52840	9.1.2	mitochondrial electron transport
SGN-U319423	AT5G52840	9.1.2	mitochondrial electron transport
SGN-U320048	AT5G52970	35.1	not assigned.no ontology
SGN-U318088	AT3G53900	23.3.1.03	nucleotide metab.salvage.phosphoribosyltransferases.upp
SGN-U317367	AT5G66120	18.5	Co-factor and vitamine metab.folate & vitamine K

SGN-U315528	AT3G04790	1.3.010, 7.2.04	OPP.non-reductive PP.ribose 5-phosphate isomerase, PS.calvin cycle.
SGN-U312398	AT3G08580	34.8	transport.metabolite transporters at the envelope membrane
SGN-U315424	AT3G54110	9.8	mitochondrial electron transport / ATP synt.uncoupling protein
SGN-U312532	AT3G50820	1.1.1.2	PS.lightreaction.photosystem II.PSII polypeptide subunits
SGN-U312449	AT4G10340	1.1.1.01	PS.lightreaction.photosystem II.LHC-II
SGN-U315383	AT2G28190	21.6	redox.dismutases and catalases
SGN-U314505	AT1G06620	21.2	redox.ascorbate and glutathione
SGN-U312641	AT5G02500	20.2.1, 29.6	stress.abiotic.heat, protein.folding
SGN-U314750	AT5G54270	1.1.1.01	PS.lightreaction.photosystem II.LHC-II
SGN-U318137	AT4G14210	16.1.4.02	secondary metab.isoprenoids.carotenoids.phytoene dehydrogenase
SGN-U312690	AT1G20340	1.1.5.01	PS.lightreaction.other electron carrier (ox/red).plastocyanin
SGN-U312571	AT1G06680	1.1.1.2	PS.lightreaction.photosystem II.PSII polypeptide subunits
SGN-U312572	AT1G06680	1.1.1.2	PS.lightreaction.photosystem II.PSII polypeptide subunits
SGN-U314262	AT1G67090	1.3.02	PS.calvin cyle.rubisco small subunit
SGN-U314701	AT5G38410	1.3.02	PS.calvin cyle.rubisco small subunit
SGN-U314722	AT5G38410	1.3.02	PS.calvin cyle.rubisco small subunit
SGN-U317633	AT1G80600	13.1.2.3.04	aa metab.synt.glutamate family.arginine.
SGN-U313361	AT5G08690	9.09	mitochondrial electron transport / ATP synt.F1-ATPase
SGN-U313676	AT4G24770	27.4	RNA.RNA binding
SGN-U313745	AT3G48560	13.1.4.1.01	aa metab.synt.branched chain group.common.acetolactate synth.
SGN-U313245	AT4G04640	1.1.04	PS.lightreaction.ATP synth.
SGN-U313693	AT4G09650	1.1.04	PS.lightreaction.ATP synth.
SGN-U316349	AT5G28540	20.2.1	stress.abiotic.heat
SGN-U312634	AT1G78380	26.09	misc.glutathione S transferases
SGN-U313713	AT3G27830	29.2.1.1.1.2.012	protein.synt.ribosomal protein.chloroplast.50S subunit.L12
SGN-U343039	ATCG00280	1.1.1.2	PS.lightreaction.photosystem II.PSII polypeptide subunits
SGN-U318409	AT5G27380	21.2.2	redox.ascorbate and glutathione.glutathione
SGN-U321015	AT2G42130	35.2	not assigned.unknown
SGN-U317769	AT2G39990	29.5.11.20	protein.degrad.ubiquitin.proteasom
SGN-U319496	AT4G18810	30.11	signalling.light
SGN-U329414	AT4G18810	30.11	signalling.light
SGN-U316006	AT1G35680	29.2.1.1.1.2.021	protein.synt.ribosomal protein.chloroplast.50S subunit.L21
SGN-U312444	AT5G66140	29.5.11.20	protein.degrad.ubiquitin.proteasom
SGN-U314700	AT5G38410	1.3.02	PS.calvin cyle.rubisco small subunit
SGN-U322688	AT2G39080	35.2	not assigned.unknown
SGN-U326961	AT3G45770	11.8.07	lipid metab.'exotics' (steroids, squalene etc)
SGN-U338507	AT2G02130	20.1	stress.biotic
SGN-U317801	AT1G51980	29.3.2	protein.targeting.mitochondria
SGN-U312808	AT1G79750	8.2.10	TCA / org. transformation.other organic acid transformaitons.malic
SGN-U312789	AT5G11670	8.2.10	TCA / org. transformation.other organic acid transformaitons.malic
SGN-U325146	AT3G20330	23.1.1.02	nucleotide metab.synt.pyrimidine.aspartate transcarbamoylase
SGN-U325988	AT3G27740	23.1.1.01	nucleotide metab.synt.pyrimidine.carbamoyl phosphate synthetase
SGN-U344034	AT4G34200	13.1.5.1.01	aa metab.synt.serine-glycine-cysteine group.serine.
SGN-U320449	AT5G39410	35.2	not assigned.unknown

SGN-U320450	AT5G39410	35.2	not assigned.unknown
SGN-U315692	AT1G33590	20.1.7	stress.biotic.PR-proteins
SGN-U327913	AT1G53520	16.8.2	secondary metab.flavonoids.chalcones
SGN-U325341	AT1G76450	1.1.1.2	PS.lightreaction.photosystem II.PSII polypeptide subunits
SGN-U315648	AT3G10670	29.8	protein assembly and cofactor ligation
SGN-U344438	AT3G51010	35.2	not assigned.unknown
SGN-U314190	AT1G23740	26.07	misc.oxidases - copper, flavone etc.
SGN-U319105	AT2G46910	31.1	cell.organisation
SGN-U317329	AT4G13200	35.2	not assigned.unknown
SGN-U315098	AT5G24400	7.1.02	OPP.oxidative PP.6-phosphogluconolactonase
SGN-U346853	AT1G32500	34.16	transport.ABC transporters and multidrug resistance systems
SGN-U316416	AT3G29320	2.2.2.02	major CHO metab.degrad.starch.starch phosphorylase
SGN-U314515	AT2G21870	9.09	mitochondrial electron transport / ATP synt.F1-ATPase
SGN-U319440	AT4G12060	29.3.99	protein.targeting.unknown
SGN-U316074	AT5G04740	13	aa metab.
SGN-U316101	AT5G60640	21.01	redox.thioredoxin
SGN-U316770	AT4G35760	35.2	not assigned.unknown
SGN-U322152	AT1G52590	35.2	not assigned.unknown
SGN-U323258	AT2G20690	18.3.02	Co-factor and vitamine metab.riboflavin.riboflavin synth.
SGN-U329804	AT3G18190	29.6	protein.folding
SGN-U320503	AT4G02530	35.1	not assigned.no ontology
SGN-U320845	AT2G37400	35.1	not assigned.no ontology
SGN-U318602	AT1G77670	16.2	secondary metab.phenylpropanoids
SGN-U314923	AT5G06290	21.05	redox.peroxiredoxin
SGN-U314924	AT5G06290	21.05	redox.peroxiredoxin
SGN-U321872	AT2G05990	11.1.06	lipid metab.FA synt. and FA elongation.enoyl ACP reductase
SGN-U314312	AT1G13440	4.09	glycolysis.glyceraldehyde 3-phosphate dehydrogenase
SGN-U313302	AT1G11840	24.02	Biodegrad. of Xenobiotics.lactoylglutathione lyase
SGN-U313499	AT2G42590	30.7	signalling.14-3-3 proteins
SGN-U316857	AT2G42590	30.7	signalling.14-3-3 proteins
SGN-U314015	AT5G65430	30.7	signalling.14-3-3 proteins
SGN-U313459	AT1G74040	13.1.4.4.01	aa metab.synt.branched chain group.
SGN-U325575	AT5G65010	13.1.3.1.01	aa metab.synt.aspartate family.asparagine.asparagine synthetase
SGN-U313514	AT5G16050	30.7	signalling.14-3-3 proteins
SGN-U314955	AT5G66190	1.1.5.03	PS.lightreaction.other electron carrier (ox/red).ferredoxin reductase
SGN-U318807	AT5G03540	31.4	cell.vesicle transport
SGN-U319060	AT5G12040	35.1	not assigned.no ontology
SGN-U321041	AT5G35170	23.4.01	nucleotide metab.phosphotransfer and pyrophosphatases.
SGN-U324835	AT5G48960	35.1	not assigned.no ontology
SGN-U313526	AT4G24830	13.1.2.3.022	aa metab.synt.glutamate family.arginine.arginosuccinate synth.
SGN-U326211	AT3G09980	35.2	not assigned.unknown
SGN-U315671	AT3G22330	27.1.2	RNA.processing.RNA helicase
SGN-U339823	AT4G27670	20.2.1	stress.abiotic.heat
SGN-U319550	AT3G01500	8.03	TCA / org. transformation.carbonic anhydrases

SGN-U323590	AT4G35090	21.6	redox.dismutases and catalases
SGN-U313481	AT3G11050	15.2	metal handling.binding, chelation and storage
SGN-U315615	AT2G47730	26.09	misc.glutathione S transferases
SGN-U333686	AT3G51260	29.5	protein.degrad.
SGN-U312411	AT4G35090	21.6	redox.dismutases and catalases
SGN-U333136	AT5G20250	3.1.2.2	minor CHO metab.raffinose family.raffinose synth.s.putative
SGN-U316872	AT1G79340	29.5	protein.degrad.
SGN-U320967	AT1G08640	35.2	not assigned.unknown
SGN-U335794	AT4G29130	2.2.1.04	major CHO metab.degrad.sucrose.hexokinase
SGN-U315143	AT4G11600	21.2.2	redox.ascorbate and glutathione.glutathione
SGN-U319272	AT4G33510	13.1.6.1.01	aa metab.synt.aromatic aa.chorismate.
SGN-U313051	AT5G06320	35.1	not assigned.no ontology
SGN-U315577	AT5G10770	27.3.99	RNA.regulation of transcription.unclassified
SGN-U317386	AT3G07020	11.8.03	lipid metab.'exotics' (steroids, squalene etc).
SGN-U317388	AT3G07020	11.8.03	lipid metab.'exotics' (steroids, squalene etc).
SGN-U317643	AT3G27890	35.1	not assigned.no ontology
SGN-U317444	AT5G13110	7.1.01	OPP.oxidative PP.G6PD
SGN-U312883	AT3G17810	23.2	nucleotide metab.degrad.
SGN-U315915	AT5G63570	19.03	tetrapyrrole synt.GSA
SGN-U315352	AT1G12000	4.05	glycolysis.pyrophosphate-fructose-6-P phosphotransferase
SGN-U315559	AT3G55800	1.3.09	PS.calvin cyle.seduheptulose bisphosphatase
SGN-U317027	AT5G64300	18.3.01	Co-factor and vitamine metab.riboflavin.GTP cyclohydrolase II
SGN-U315549	AT3G62410	35.1	not assigned.no ontology
SGN-U320540	AT1G47710	29.5.5	protein.degrad.serine protease
SGN-U330760	AT5G53480	29.3.1	protein.targeting.nucleus
SGN-U327265	AT4G26900	13.1.7.010	aa metab.synt.histidine.Imidazole glycerol phosphate synth.
SGN-U314149	AT3G63140	27.3.99	RNA.regulation of transcription.unclassified
SGN-U315697	AT2G05990	11.1.06	lipid metab.FA synt. and FA elongation.enoyl ACP reductase
SGN-U314582	AT5G53560	35.1	not assigned.no ontology
SGN-U312544	AT2G39730	1.3.013	PS.calvin cyle.rubisco interacting
SGN-U321652	AT3G10405	35.2	not assigned.unknown
SGN-U320459	AT3G19420	29.4	protein.postranslational modification
SGN-U314139	AT5G60600	16.1.1.06	secondary metab.isoprenoids.non-mevalonate pathway.HDS
SGN-U312375	AT4G30920	29.5	protein.degrad.
SGN-U313542	AT4G20260	35.1	not assigned.no ontology
SGN-U328875	AT4G09020	2.1.2.04	major CHO metab.synt.starch.debranching
SGN-U333011	AT4G09020	2.1.2.04	major CHO metab.synt.starch.debranching
SGN-U323261	AT5G04140	12.2.1.01	N-metab.ammonia metab.glutamate synth..ferredoxin dependent
SGN-U330538	AT1G61800	34.8	transport.metabolite transporters at the envelope membrane
SGN-U314772	AT5G63310	23.4.010	nucleotide metab.phosphotransfer and pyrophosphatases.
SGN-U314773	AT5G63310	23.4.010	nucleotide metab.phosphotransfer and pyrophosphatases.
SGN-U315094	AT1G78900	34.1	transport.p- and v-ATPases
SGN-U336151	AT5G65770	29.5	protein.degrad.
SGN-U317202	AT3G01480	29.6	protein.folding

SGN-U315147	AT4G33010	13.2.5.2	aa metab.degrad.serine-glycine-cysteine group.glycine
SGN-U318116	AT4G33010	13.2.5.2	aa metab.degrad.serine-glycine-cysteine group.glycine
SGN-U328612	AT5G26570	2.2.2.03	major CHO metab.degrad.starch.glucan water dikinase
SGN-U317261	AT5G16390	11.1.01	lipid metab.FA synt. and FA elongation.Acetyl CoA Carboxylation
SGN-U315145	AT3G42050	34.1.01	transport.p- and v-ATPases.H+-transporting two-sector ATPase
SGN-U315146	AT3G42050	34.1.01	transport.p- and v-ATPases.H+-transporting two-sector ATPase
SGN-U325875	AT1G74960	11.1.03	lipid metab.FA synt. and FA elongation.ketoacyl ACP synth.
SGN-U316868	AT1G62640	11.1.03	lipid metab.FA synt. and FA elongation.ketoacyl ACP synth.
SGN-U335865	AT1G62640	11.1.03	lipid metab.FA synt. and FA elongation.ketoacyl ACP synth.
SGN-U315367	AT3G22370	9.04	mitochondrial electron transport / ATP synt.alternative oxidase
SGN-U315474	AT5G46290	11.1.03	lipid metab.FA synt. and FA elongation.ketoacyl ACP synth.
SGN-U315475	AT5G46290	11.1.03	lipid metab.FA synt. and FA elongation.ketoacyl ACP synth.
SGN-U314254	AT1G67090	1.3.02	PS.calvin cyle.rubisco small subunit
SGN-U322816	AT5G64860	2.2.2.04	major CHO metab.degrad.starch.D enzyme
SGN-U333138	AT5G64860	2.2.2.04	major CHO metab.degrad.starch.D enzyme
SGN-U317344	AT1G31230	13.1.3.6.1.01	aa metab.synt.aspartate family.misc.homoserine.aspartate kinase
SGN-U316922	AT1G20510	16.2	secondary metab.phenylpropanoids
SGN-U314260	AT1G31330	1.1.2.2	PS.lightreaction.photosystem I.PSI polypeptide subunits
SGN-U314747	AT1G07790	28.1.3	DNA.synt./chromatin structure.histone
SGN-U313132	AT1G07790	28.1.3	DNA.synt./chromatin structure.histone
SGN-U315486	AT5G03290	8.2.04	TCA / org. transformation.other organic acid transformaitons.IDH
SGN-U315487	AT5G03290	8.2.04	TCA / org. transformation.other organic acid transformaitons.IDH
SGN-U313875	AT2G33040	9.09	mitochondrial electron transport / ATP synt.F1-ATPase
SGN-U312610	AT2G05710	8.1.03	TCA / org. transformation.TCA.aconitase
SGN-U320721	AT2G04030	20.2.1	stress.abiotic.heat
SGN-U319774	AT2G04350	11.1.09	lipid metab.FA synt. and FA elongation.long chain fatty acid CoA ligase
SGN-U313979	AT1G02205	16.7	secondary metab.wax
SGN-U324140	AT2G12400	35.2	not assigned.unknown
SGN-U321911	AT2G14260	29.5	protein.degrad.
SGN-U318650	AT1G06130	24.01	Biodegrad. of Xenobiotics.hydroxyacylglutathione hydrolase
SGN-U344079	AT2G16890	26.2	misc.UDP glucosyl and glucoronyl transferases
SGN-U318911	AT3G07100	29.3.4.2	protein.targeting.secretory pathway.golgi
SGN-U318557	AT2G22480	4.04	glycolysis.PPFK
SGN-U316796	AT1G11360	20.2.99	stress.abiotic.unspecified
SGN-U321053	AT1G11360	20.2.99	stress.abiotic.unspecified
SGN-U322514	AT1G11940	35.2	not assigned.unknown
SGN-U312724	AT3G14420	1.2.02	PS.photorespiration.glycolate oxydase
SGN-U317890	AT5G13800	35.1	not assigned.no ontology
SGN-U314095	AT1G16890	29.5.11.03	protein.degrad.ubiquitin.E2
SGN-U319280	AT5G17530	4.02	glycolysis.PGM
SGN-U316715	AT4G27270	11.8	lipid metab.'exotics' (steroids, squalene etc)
SGN-U324911	AT1G23180	35.1.3	not assigned.armadillo/beta-catenin repeat family protein
SGN-U319497	AT2G43945	35.2	not assigned.unknown
SGN-U318328	AT5G41670	7.1.03	OPP.oxidative PP.6-phosphogluconate dehydrogenase

SGN-U316498	AT1G50200	29.1.07	protein.aa activation.alanine-tRNA ligase
SGN-U325976	AT3G60370	29.6	protein.folding
SGN-U318052	AT1G53000	35.1	not assigned.no ontology
SGN-U320456	AT5G49970	35.1	not assigned.no ontology
SGN-U318192	AT5G60640	21.01	redox.thioredoxin
SGN-U334288	AT1G67700	29.5	protein.degrad.
SGN-U315992	AT5G62810	35.1	not assigned.no ontology
SGN-U321324	AT5G64370	23.2	nucleotide metab.degrad.
SGN-U318255	AT1G77090	1.1.1.2	PS.lightreaction.photosystem II.PSII polypeptide subunits
SGN-U321266	AT1G80030	20.2.1	stress.abiotic.heat
SGN-U313057	AT1G65980	21.05	redox.peroxiredoxin
SGN-U326999	AT5G14220	19.09	tetrapyrrole synt.protoporphyrin IX oxidase
SGN-U325849	AT3G29320	2.2.2.02	major CHO metab.degrad.starch.starch phosphorylase
SGN-U333374	AT3G29320	2.2.2.02	major CHO metab.degrad.starch.starch phosphorylase
SGN-U345057	AT3G29320	2.2.2.02	major CHO metab.degrad.starch.starch phosphorylase
SGN-U318401	AT3G06350	13.1.6.1.010	aa metab.synt.aromatic aa.
SGN-U314964	AT5G17330	13.1.1.1.01	aa metab.synt.central aa metab.GABA.Glutamate decarboxylase
SGN-U315478	AT1G64520	29.5.11.20	protein.degrad.ubiquitin.proteasom
SGN-U315116	AT1G10760	2.2.2.03	major CHO metab.degrad.starch.glucan water dikinase
SGN-U316181	AT2G43030	29.2.1.1.1.2.03	protein.synt.ribosomal protein.chloroplast.50S subunit.L3
SGN-U317911	AT5G13510	29.2.1.1.1.2.010	protein.synt.ribosomal protein.chloroplast.50S subunit.L10
SGN-U313176	AT1G56190	1.3.03	PS.calvin cyle.phosphoglycerate kinase
SGN-U317540	AT5G04140	12.2.1.01	N-metab.ammonia metab.glutamate synth.ferredoxin dependent
SGN-U322093	AT2G44050	18.3.02	Co-factor and vitamine metab.riboflavin.riboflavin synth.
SGN-U315384	AT2G28190	21.6	redox.dismutases and catalases
SGN-U313819	AT4G25100	21.6	redox.dismutases and catalases
SGN-U315448	AT2G20890	29.3	protein.targeting
SGN-U317572	AT5G10330	13.1.7.06	aa metab.synt.histidine.histidinol-phosphate aminotransferase
SGN-U314161	AT5G65020	31.1	cell.organisation
SGN-U313907	AT1G35720	31.1	cell.organisation
SGN-U313378	AT1G63770	29.5	protein.degrad.
SGN-U312985	AT1G32470	1.2.04.04	PS.photorespiration.glycine cleavage.H protein
SGN-U324031	AT2G44920	35.1	not assigned.no ontology
SGN-U312359	AT3G20970	29.8	protein assembly and cofactor ligation
SGN-U316684	AT2G33430	33.99	development.unspecified
SGN-U317180	AT2G29990	9.2.1	mitochondrial electron transport / ATP synt.NADH-DH.type II.
SGN-U322461	AT2G29080	29.5.09	protein.degrad.AAA type
SGN-U312320	AT2G45290	1.3.08, 7.2.01	OPP.non-reductive PP.transketolase, PS.calvin cyle.transketolase
SGN-U312322	AT3G60750	1.3.08, 7.2.01	PS.calvin cyle.transketolase, OPP.non-reductive PP.transketolase
SGN-U323721	AT3G60750	1.3.08, 7.2.01	PS.calvin cyle.transketolase, OPP.non-reductive PP.transketolase
SGN-U314396	AT3G60820	29.5.11.20	protein.degrad.ubiquitin.proteasom
SGN-U313362	AT5G08690	9.09	mitochondrial electron transport / ATP synt.F1-ATPase
SGN-U312858	AT4G03280	1.1.03	PS.lightreaction.cytochrome b6/f
SGN-U314329	AT5G04590	14.03	S-assimilation.sulfite redox

SGN-U320197	AT5G53490	35.1	not assigned.no ontology
SGN-U313369	AT3G48890	21.2	redox.ascorbate and glutathione
SGN-U313370	AT3G48890	21.2	redox.ascorbate and glutathione
SGN-U319083	AT5G46800	34.9	transport.
SGN-U313711	AT5G65430	30.7	signalling.14-3-3 proteins
SGN-U316154	AT4G35260	8.1.04	TCA / org. transformation.TCA.IDH
SGN-U333873	AT3G14420	1.2.02	PS.photorespiration.glycolate oxydase
SGN-U316716	AT5G06140	35.1	not assigned.no ontology
SGN-U313706	AT1G72330	13.1.1.3.01	aa metab.synt.central aa metab.alanine.alanine aminotransferase
SGN-U326692	AT3G55010	23.1.2.05	nucleotide metab.synt.purine.AIR synth.
SGN-U312779	AT2G37690	23.1.2.06	nucleotide metab.synt.purine.AIR carboxylase
SGN-U323045	AT3G21110	23.1.2.07	nucleotide metab.synt.purine.SAICAR synthetase
SGN-U318064	AT3G57610	23.1.2.20	nucleotide metab.synt.purine.adenylosuccinate synth.
SGN-U318065	AT3G57610	23.1.2.20	nucleotide metab.synt.purine.adenylosuccinate synth.
SGN-U317502	AT3G20390	35.1	not assigned.no ontology
SGN-U319833	AT1G09830	23.1.2.02	nucleotide metab.synt.purine.GAR Synthetase
SGN-U313450	AT1G74470	16.1.1	secondary metab.isoprenoids.non-mevalonate pathway
SGN-U315589	AT4G01900	30.1.01	signalling.in sugar and nutrient physiology
SGN-U316850	AT5G63840	26.03	misc.gluco-, galacto- and mannosidases
SGN-U312728	AT5G50850	8.1.01.01	TCA / org. transformation.TCA.pyruvate DH.E1
SGN-U315359	AT5G50850	8.1.01.01	TCA / org. transformation.TCA.pyruvate DH.E1
SGN-U314064	AT1G66430	2.2.1.01	major CHO metab.degrad.sucrose.fructokinase
SGN-U332166	AT5G02100	31.4	cell.vesicle transport
SGN-U319404	AT3G23400	31.1	cell.organisation
SGN-U317564	AT4G02610	13.1.6.5.05	aa metab.synt.aromatic aa.tryptophan.tryptophan synth.
SGN-U317144	AT4G23100	21.2.2	redox.ascorbate and glutathione.glutathione
SGN-U313789	AT4G32260	1.1.04	PS.lightreaction.ATP synth.
SGN-U312699	AT1G01620	34.19.1	transport.Major Intrinsic Proteins.PIP
SGN-U333847	AT1G01620	34.19.1	transport.Major Intrinsic Proteins.PIP
SGN-U312782	AT5G50920	29.5.5	protein.degrad.serine protease
SGN-U312784	AT5G50920	29.5.5	protein.degrad.serine protease
SGN-U312783	AT5G50920	29.5.5	protein.degrad.serine protease
SGN-U325141	AT2G22170	35.1	not assigned.no ontology
SGN-U314517	AT5G35630	12.2.02	N-metab.ammonia metab.glutamine synth.
SGN-U314177	AT4G02340	26.01	misc.misc2
SGN-U313391	AT1G48850	13.1.6.1.07	aa metab.synt.aromatic aa.chorismate.chorismate synth.
SGN-U317701	AT5G36880	11.1.08	lipid metab.FA synt. and FA elongation.acyl coa ligase
SGN-U327405	AT2G40840	2.2.2.04	major CHO metab.degrad.starch.D enzyme
SGN-U324102	AT2G29720	26.07	misc.oxidases - copper, flavone etc.
SGN-U316710	AT1G56050	30.5	signalling.G-proteins
SGN-U321941	AT1G63610	35.2	not assigned.unknown
SGN-U325741	AT4G14570	29.5	protein.degrad.
SGN-U339328	AT4G30310	3.3	minor CHO metab.sugar alcohols
SGN-U319834	AT5G36210	29.5	protein.degrad.

SGN-U313379	AT1G63770	29.5	protein.degrad.
SGN-U331682	AT4G28706	3.5	minor CHO metab.others
SGN-U318011	AT4G38225	35.2	not assigned.unknown
SGN-U316105	AT4G02930	29.2.4	protein.synt.elongation
SGN-U314061	AT3G26060	21.05	redox.peroxiredoxin
SGN-U315455	AT3G54660	21.2.2	redox.ascorbate and glutathione.glutathione
SGN-U335161	AT3G54660	21.2.2	redox.ascorbate and glutathione.glutathione
SGN-U313929	AT2G30950	29.5.07	protein.degrad.metalloprotease
SGN-U346451	AT2G03390	28.1	DNA.synt./chromatin structure
SGN-U320482	AT4G00570	8.2.10	TCA / org. transformation.other organic acid transformaitons.malic
SGN-U315084	AT4G37930	25.01	C1-metab.glycine hydroxymethyltransferase
SGN-U323843	AT2G13560	8.2.10	TCA / org. transformation.other organic acid transformaitons.malic
SGN-U313664	AT1G11860	13.2.5.2	aa metab.degrad.serine-glycine-cysteine group.glycine
SGN-U320318	AT1G79230	13.2.5.3	aa metab.degrad.serine-glycine-cysteine group.cysteine
SGN-U320732	AT1G79230	13.2.5.3	aa metab.degrad.serine-glycine-cysteine group.cysteine
SGN-U338973	AT5G38410	1.3.02	PS.calvin cyle.rubisco small subunit
SGN-U343085	AT1G13440	4.09	glycolysis.glyceraldehyde 3-phosphate dehydrogenase
SGN-U321010	AT3G66654	31.3.01	cell.cycle.peptidylprolyl isomerase
SGN-U317077	AT5G58140	29.4	protein.postranslational modification
SGN-U320525	AT3G48110	29.1.014	protein.aa activation.glycine-tRNA ligase
SGN-U321715	AT5G64090	35.2	not assigned.unknown
SGN-U316424	AT3G54050	1.3.07	PS.calvin cyle.FBPase
SGN-U312392	AT4G20360	29.2.4	protein.synt.elongation
SGN-U321085	AT3G44890	29.2.1.1.1.2.09	protein.synt.ribosomal protein.chloroplast.50S subunit.L9
SGN-U345138	AT1G63940	21.2.1	redox.ascorbate and glutathione.ascorbate
SGN-U313810	AT4G24190	20.2.1	stress.abiotic.heat
SGN-U325914	AT4G36810	16.1.1.010	secondary metab.isoprenoids.
SGN-U317524	AT2G15620	12.1.02	N-metab.nitrate metab.nitrite reductase
SGN-U313930	AT5G01410	35.1	not assigned.no ontology
SGN-U320746	AT5G48440	35.1	not assigned.no ontology
SGN-U319456	AT3G63190	31.2	cell.division
SGN-U312675	AT1G21750	21.01	redox.thioredoxin
SGN-U318204	AT1G43800	11.1.015	lipid metab.FA synt. and FA elongation.ACP desaturase
SGN-U320588	AT5G55280	31.2	cell.division
SGN-U315306	AT1G59900	8.1.01.01	TCA / org. transformation.TCA.pyruvate DH.E1
SGN-U324953	AT2G12400	35.2	not assigned.unknown
SGN-U316403	AT5G26030	19.020	tetrapyrrole synt.ferrochelatase
SGN-U319524	AT1G77550	31.1	cell.organisation
SGN-U313496	AT3G22890	14.01	S-assimilation.APS
SGN-U313497	AT3G22890	14.01	S-assimilation.APS
SGN-U312832	AT1G21750	21.01	redox.thioredoxin
SGN-U316028	AT1G77510	21.01	redox.thioredoxin
SGN-U326739	AT1G77510	21.01	redox.thioredoxin
SGN-U313292	AT2G18110	29.2.4	protein.synt.elongation

SGN-U334255	AT2G18110	29.2.4	protein.synt.elongation
SGN-U314213	AT3G52300	9.09	mitochondrial electron transport / ATP synt.F1-ATPase
SGN-U312608	AT4G38970	1.3.06	PS.calvin cyle.aldolase
SGN-U312609	AT4G38970	1.3.06	PS.calvin cyle.aldolase
SGN-U312344	AT4G38970	1.3.06	PS.calvin cyle.aldolase
SGN-U313534	AT3G22960	11.1.030	lipid metab.FA synt. and FA elongation.pyruvate kinase
SGN-U320512	AT1G32440	11.1.030	lipid metab.FA synt. and FA elongation.pyruvate kinase
SGN-U320146	AT5G23750	27.3.99	RNA.regulation of transcription.unclassified
SGN-U321893	AT2G17265	13.1.3.6.1.04	aa metab.synt.aspartate family.misc.homoserine.homoserine kinase
SGN-U314897	AT3G16640	35.1	not assigned.no ontology
SGN-U314911	AT3G16640	35.1	not assigned.no ontology
SGN-U316763	Gi|49525850	expressed protein	
SGN-U322690	AT1G26090	35.2	not assigned.unknown
SGN-U314092	AT4G08390	21.2.1	redox.ascorbate and glutathione.ascorbate
SGN-U322081	AT1G26160	35.1	not assigned.no ontology
SGN-U340973	AT5G63060	34.99	transport.misc
SGN-U314873	AT5G57850	26.26.1	misc.aminotransferases.aminotransferase class IV family protein
SGN-U326257	AT1G52340	17.1.1.1.011	hormone metab.abscisic acid.synt.-degrad.synt.short chain ADH
SGN-U325252	AT4G25450	34.16	transport.ABC transporters and multidrug resistance systems
SGN-U316258	AT4G15560	16.1.1.01	secondary metab.isoprenoids.non-mevalonate pathway.DXS
SGN-U315096	AT5G24400	7.1.02	OPP.oxidative PP.6-phosphogluconolactonase
SGN-U318386	AT5G24400	7.1.02	OPP.oxidative PP.6-phosphogluconolactonase
SGN-U314835	AT3G07480	35.2	not assigned.unknown
SGN-U316307	AT5G53560	35.1	not assigned.no ontology
SGN-U322316	AT4G26670	29.3.2	protein.targeting.mitochondria
SGN-U318008	AT4G33650	26.17	misc.dynamin
SGN-U325296	AT3G55200	27.1.1	RNA.processing.splicing
SGN-U314448	AT3G52960	21.05	redox.peroxiredoxin
SGN-U312791	AT1G32060	1.3.012	PS.calvin cyle.PRK
SGN-U315579	AT4G38100	29.5	protein.degrad.
SGN-U315899	AT1G48420	17.5.1	hormone metab.ethylene.synt.-degrad.
SGN-U320170	AT5G65840	35.2	not assigned.unknown
SGN-U323083	AT2G42520	27.1.2	RNA.processing.RNA helicase
SGN-U318269	AT2G47730	26.09	misc.glutathione S transferases
SGN-U316544	AT3G54900	34.21	transport.calcium
SGN-U315110	AT1G24360	11.1.04	lipid metab.FA synt. and FA elongation.ACP oxoacyl reductase
SGN-U316185	AT1G32220	35.2	not assigned.unknown
SGN-U312962	AT5G24650	29.3.2	protein.targeting.mitochondria
SGN-U315769	AT1G29150	29.5.11.20	protein.degrad.ubiquitin.proteasom
SGN-U315009	AT2G43180	35.2	not assigned.unknown
SGN-U324954	AT4G23060	30.3	signalling.calcium
SGN-U316793	AT5G19940	31.1	cell.organisation
SGN-U319182	AT4G25550	35.2	not assigned.unknown
SGN-U313307	AT5G13120	31.3.01	cell.cycle.peptidylprolyl isomerase

SGN-U329585	AT3G03710	27.1	RNA.processing
SGN-U324342	AT5G43100	27.3.99	RNA.regulation of transcription.unclassified
SGN-U317056	AT5G63620	35.1	not assigned.no ontology
SGN-U317869	AT5G26830	29.1.03	protein.aa activation.threonine-tRNA ligase
SGN-U322404	AT1G54500	29.8	protein assembly and cofactor ligation
SGN-U319207	AT2G39290	11.3	lipid metab.Phospholipid synt.
SGN-U313562	AT3G19820	17.3.1.2.08	hormone metab.brassinosteroid.synt.-degrad.sterols.DWF1
SGN-U317631	AT1G53670	27.3.67	RNA.regulation of transcription.putative transcription regulator
SGN-U312438	AT2G05100	1.1.1.01	PS.lightreaction.photosystem II.LHC-II
SGN-U320649	AT3G47520	8.2.09	TCA / org. transformation.other organic acid transformaitons.cyt MDH
SGN-U320831	AT3G47520	8.2.09	TCA / org. transformation.other organic acid transformaitons.cyt MDH
SGN-U317897	AT4G24620	4.03	glycolysis.G6PIsomerase
SGN-U312871	AT4G05180	1.1.1.2	PS.lightreaction.photosystem II.PSII polypeptide subunits
SGN-U315202	AT4G39730	35.1	not assigned.no ontology
SGN-U316834	AT5G24490	29.2.1.1.1.1.0530	protein.synt.ribosomal protein.chloroplast.30S subunit.S30A
SGN-U315485	AT3G01280	34.20	transport.porins
SGN-U326722	AT1G80480	31.1	cell.organisation
SGN-U315027	AT3G01280	34.20	transport.porins
SGN-U314012	AT3G12260	35.1	not assigned.no ontology
SGN-U316751	AT2G44530	23.1.03	nucleotide metab.synt.PRS-PP
SGN-U330586	AT5G54110	31.4	cell.vesicle transport
SGN-U333115	AT4G25450	34.16	transport.ABC transporters and multidrug resistance systems
SGN-U325887	AT1G20650	29.4.1.57	protein.postranslational modification.kinase.
SGN-U326864	AT4G39120	3.4.05	minor CHO metab.myo-inositol.inositol phosphatase
SGN-U319102	AT5G66090	35.2	not assigned.unknown
SGN-U321113	AT3G06850	13.2.4.1	aa metab.degrad.branched-chain group.shared
SGN-U326597	AT1G18270	35.1	not assigned.no ontology
SGN-U334708	AT5G58330	8.2.09	TCA / org. transformation.other organic acid transformaitons.cyt MDH
SGN-U317020	AT1G70710	26.03	misc.gluco-, galacto- and mannosidases
SGN-U316444	AT5G45390	29.5.5	protein.degrad.serine protease
SGN-U323759	AT1G20630	21.6	redox.dismutases and catalases
SGN-U312533	AT1G07040	35.2	not assigned.unknown
SGN-U326817	AT1G69830	2.2.2.1	major CHO metab.degrad.starch.starch cleavage
SGN-U316899	AT3G02900	35.2	not assigned.unknown
SGN-U320886	AT1G53280	35.1	not assigned.no ontology
SGN-U314614	AT3G15000	35.1	not assigned.no ontology
SGN-U314615	AT3G15000	35.1	not assigned.no ontology
SGN-U314024	AT5G48230	16.1.2.01	secondary metab.isoprenoids.mevalonate pathway.
SGN-U317217	AT4G33030	11.10.03	lipid metab.glycolipid synt.UDP-sulfoquinovose synth.
SGN-U316449	AT5G23120	29.8	protein assembly and cofactor ligation
SGN-U316707	AT1G73390	35.2	not assigned.unknown
SGN-U315986	AT4G08900	13.2.2.3	aa metab.degrad.glutamate family.arginine
SGN-U315987	AT4G08900	13.2.2.3	aa metab.degrad.glutamate family.arginine
SGN-U316271	AT5G16620	29.3.3	protein.targeting.chloroplast

SGN-U312324	AT2G43950	35.2	not assigned.unknown
SGN-U313151	AT3G18035	28.1.3	DNA.synt./chromatin structure.histone
SGN-U329463	AT1G03680	21.01	redox.thioredoxin
SGN-U329427	AT5G67630	28.1	DNA.synt./chromatin structure
SGN-U327931	AT3G49050	30.3	signalling.calcium
SGN-U314443	AT4G30620	35.2	not assigned.unknown
SGN-U313315	AT5G18670	2.2.2.1	major CHO metab.degrad.starch.starch cleavage
SGN-U313592	AT1G70410	8.03	TCA / org. transformation.carbonic anhydrases
SGN-U313593	AT5G14740	8.03	TCA / org. transformation.carbonic anhydrases
SGN-U312799	AT5G14780	25.10	C1-metab.formate dehydrogenase
SGN-U313989	AT5G58330	8.2.09	TCA / org. transformation.other organic acid transformaitons.cyt MDH
SGN-U317095	AT2G22250	13.1.1.2.01	aa metab.synt.central aa metab.aspartate.aspartate aminotransferase
SGN-U312785	AT2G33800	29.2.1.1.1.1.05	protein.synt.ribosomal protein.chloroplast.30S subunit.S5
SGN-U323581	AT5G14590	8.1.04	TCA / org. transformation.TCA.IDH
SGN-U317104	AT1G12520	21.6	redox.dismutases and catalases
SGN-U330850	AT2G25840	29.1	protein.aa activation
SGN-U321940	AT3G06350	13.1.6.1.010	aa metab.synt.aromatic aa.chorismate.
SGN-U312554	AT5G42270	29.5.07	protein.degrad.metalloprotease
SGN-U319447	AT3G10350	34.18.01	transport.unspecified anions.arsenite-transporting ATPase
SGN-U314429	AT5G17230	16.1.4.01	secondary metab.isoprenoids.carotenoids.phytoene synth.
SGN-U314440	AT3G02090	29.3.2	protein.targeting.mitochondria
SGN-U338687	AT3G02090	29.3.2	protein.targeting.mitochondria
SGN-U316731	AT5G42080	30.5	signalling.G-proteins
SGN-U343310	AT5G42080	30.5	signalling.G-proteins
SGN-U321296	AT3G11130	31.4	cell.vesicle transport
SGN-U346835	AT3G11130	31.4	cell.vesicle transport
SGN-U317026	AT1G11750	29.5.5	protein.degrad.serine protease
SGN-U312768	AT5G67500	34.20	transport.porins
SGN-U312769	AT5G67500	34.20	transport.porins
SGN-U329431	AT3G52170	27.3.67	RNA.regulation of transcription.putative transcription regulator
SGN-U317062	AT2G22780	6.03	gluconeogenesis.Malate DH
SGN-U317456	AT1G69830	2.2.2.1	major CHO metab.degrad.starch.starch cleavage
SGN-U317915	AT4G04770	29.8	protein assembly and cofactor ligation
SGN-U315593	AT3G29590	16.8.1.021	secondary metab.flavonoids.anthocyanins.
SGN-U323169	AT2G38040	16.99	secondary metab.unspecified
SGN-U314358	AT1G77120	5.03	fermentation.ADH
SGN-U314361	AT1G77120	5.03	fermentation.ADH
SGN-U321417	AT1G05810	30.5	signalling.G-proteins
SGN-U312581	AT1G07890	21.2.1	redox.ascorbate and glutathione.ascorbate
SGN-U315464	AT3G48170	16.4.2.01	secondary metab.N misc.betaine.betaine-aldehyde dehydrogenase
SGN-U313095	AT2G36830	34.19.2	transport.Major Intrinsic Proteins.TIP
SGN-U341277	AT2G38310	35.1	not assigned.no ontology
SGN-U317343	AT5G64050	29.1.017	protein.aa activation.glutamate-tRNA ligase
SGN-U316479	AT2G35490	31.1	cell.organisation

SGN-U316997	AT5G65780	13.1.4.1.04	aa metab.synt.branched chain group.common.
SGN-U316996	AT5G65780	13.1.4.1.04	aa metab.synt.branched chain group.common.
SGN-U313447	AT2G20260	1.1.2.2	PS.lightreaction.photosystem I.PSI polypeptide subunits
SGN-U316291	AT1G11360	20.2.99	stress.abiotic.unspecified
SGN-U321444	AT3G51140	35.2	not assigned.unknown
SGN-U316492	AT3G15730	11.9.3.01	lipid metab.lipid degrad.lysophospholipases.phospholipase D
SGN-U320566	AT3G63130	30.5	signalling.G-proteins
SGN-U317763	AT4G09010	21.2.1	redox.ascorbate and glutathione.ascorbate
SGN-U314086	AT4G25130	29.4	protein.postranslational modification
SGN-U313537	AT1G19570	21.2.1	redox.ascorbate and glutathione.ascorbate
SGN-U313719	AT5G16710	21.2.1	redox.ascorbate and glutathione.ascorbate
SGN-U341182	AT1G73990	29.5	protein.degrad.
SGN-U328823	AT1G47840	2.2.1.04	major CHO metab.degrad.sucrose.hexokinase
SGN-U315861	AT5G61790	30.3	signalling.calcium
SGN-U324386	AT1G18700	20.2.1	stress.abiotic.heat
SGN-U319020	AT3G52380	27.4	RNA.RNA binding
SGN-U314093	AT1G77490	21.2.1	redox.ascorbate and glutathione.ascorbate
SGN-U313237	AT5G19760	34.9	transport.
SGN-U316068	AT4G22920	35.2	not assigned.unknown
SGN-U312391	AT4G20360	29.2.4	protein.synt.elongation
SGN-U320127	AT4G01150	35.2	not assigned.unknown
SGN-U314101	AT5G15450	20.2.1	stress.abiotic.heat
SGN-U314096	AT1G78870	29.5.11.03	protein.degrad.ubiquitin.E2
SGN-U316277	AT4G35770	33.99	development.unspecified
SGN-U341662	AT3G02760	29.1.021	protein.aa activation.histidine-tRNA ligase
SGN-U318043	AT2G12190	26.10	misc.cytochrome P450
SGN-U320487	AT1G63940	21.2.1	redox.ascorbate and glutathione.ascorbate
SGN-U318220	AT5G22330	35.1	not assigned.no ontology
SGN-U321435	AT4G13550	11.9.2.01	lipid metab.lipid degrad.lipases.triacylglycerol lipase
SGN-U319980	AT4G25280	23.4.3	nucleotide metab.phosphotransfer and pyrophosphatases
SGN-U312553	AT4G35230	29.4.1.52	protein.postranslational modification.kinase
SGN-U312976	AT5G09590	20.2.1	stress.abiotic.heat
SGN-U312977	AT5G09590	20.2.1	stress.abiotic.heat
SGN-U312669	AT5G20720	29.6	protein.folding
SGN-U315344	AT5G20720	29.6	protein.folding
SGN-U313800	AT3G48000	5.10	fermentation.aldehyde dehydrogenase
SGN-U312586	AT1G07890	21.2.1	redox.ascorbate and glutathione.ascorbate
SGN-U312978	AT4G09320	23.4.010	nucleotide metab.phosphotransfer and pyrophosphatases.
SGN-U319168	AT2G19940	35.1	not assigned.no ontology
SGN-U319839	AT1G14610	29.1.09	protein.aa activation. valine-tRNA ligase
SGN-U317750	AT5G43070	35.1	not assigned.no ontology
SGN-U317751	AT5G43070	35.1	not assigned.no ontology
SGN-U312731	AT1G74970	29.2.1.1.1.1.09	protein.synt.ribosomal protein.chloroplast.30S subunit.S9
SGN-U315458	AT5G37510	9.1.2	mitochondrial electron transport / ATP synt.NADH-DH.

SGN-U318786	AT1G63970	16.1.1.05	secondary metab.isoprenoids.non-mevalonate pathway.MCS
SGN-U318693	AT5G53530	29.3.4.3	protein.targeting.secretory pathway.vacuole
SGN-U322417	AT3G55440	4.08	glycolysis.TPI
SGN-U312604	AT1G49760	27.1	RNA.processing
SGN-U315905	AT1G49760	27.1	RNA.processing
SGN-U319339	AT5G42650	17.7.1.03	hormone metab.jasmonate.synt.-degrad.allene oxidase synth.
SGN-U314789	AT4G13010	26.07	misc.oxidases - copper, flavone etc.
SGN-U316949	AT1G79210	29.5.11.20	protein.degrad.ubiquitin.proteasom
SGN-U316950	AT1G16470	29.5	protein.degrad.
SGN-U312802	AT1G42970	1.3.04	PS.calvin cyle.GAP
SGN-U312804	AT1G42970	1.3.04	PS.calvin cyle.GAP
SGN-U334503	AT3G52990	4.013	glycolysis.PK
SGN-U324120	AT5G22620	35.1	not assigned.no ontology
SGN-U315366	AT4G31480	31.4	cell.vesicle transport
SGN-U316627	AT5G50370	23.4.01	nucleotide metab.phosphotransfer and pyrophosphatases.
SGN-U316600	AT3G26710	35.2	not assigned.unknown
SGN-U318939	AT3G10300	30.3	signalling.calcium
SGN-U332370	AT5G06410	20.2.1	stress.abiotic.heat
SGN-U316708	AT3G58140	29.1.020	protein.aa activation.phenylalanine-tRNA ligase
SGN-U319329	AT3G47860	35.1	not assigned.no ontology
SGN-U312987	AT3G55440	4.08	glycolysis.TPI
SGN-U312988	AT3G55440	4.08	glycolysis.TPI
SGN-U314787	AT2G01140	1.3.06	PS.calvin cyle.aldolase
SGN-U314730	AT1G68070	29.5.11.04.02	protein.degrad.ubiquitin.E3.RING
SGN-U312698	AT1G01620	34.19.1	transport.Major Intrinsic Proteins.PIP
SGN-U330351	AT2G37500	13.1.2.3,13.1.2.3.02	aa metab.synt.glutamate family.arginine, aa metab.
SGN-U313482	AT3G11050	15.2	metal handling.binding, chelation and storage
SGN-U312814	AT2G37170	34.19.1	transport.Major Intrinsic Proteins.PIP
SGN-U326776	AT4G14570	29.5	protein.degrad.
SGN-U327002	AT2G43400	9.3	mitochondrial electron transport
SGN-U322181	AT3G20920	35.1	not assigned.no ontology
SGN-U341094	AT3G54440	26.03	misc.gluco-, galacto- and mannosidases
SGN-U317396	AT1G09130	29.5.5	protein.degrad.serine protease
SGN-U332028	AT1G18700	20.2.1	stress.abiotic.heat
SGN-U315754	AT2G42210	29.3.2	protein.targeting.mitochondria
SGN-U315755	AT2G42210	29.3.2	protein.targeting.mitochondria
SGN-U320939	AT4G00026	35.2	not assigned.unknown
SGN-U316215	AT4G20980	29.2.3	protein.synt.initiation
SGN-U319675	AT4G29060	29.2.4	protein.synt.elongation
SGN-U327412	AT1G31780	35.1	not assigned.no ontology
SGN-U315881	AT4G19880	26.09	misc.glutathione S transferases
SGN-U317905	AT2G16950	29.3.1	protein.targeting.nucleus
SGN-U315206	AT4G31990	13.1.1.2.01	aa metab.synt.central aa metab.aspartate.aspartate aminotransferase
SGN-U313214	AT1G29930	1.1.1.01	PS.lightreaction.photosystem II.LHC-II

SGN-U314198	AT2G04520	29.2.3	protein.synt.initiation
SGN-U313338	AT4G34350	16.1.1.07	secondary metab.isoprenoids.non-mevalonate pathway.HDR
SGN-U318394	AT1G76020	35.2	not assigned.unknown
SGN-U327158	AT1G76020	35.2	not assigned.unknown
SGN-U314372	AT2G17800	30.5	signalling.G-proteins
SGN-U312843	AT1G61520	1.1.2.01	PS.lightreaction.photosystem I.LHC-I
SGN-U318309	AT3G03890	35.2	not assigned.unknown
SGN-U324006	AT5G51820	4.02	glycolysis.PGM
SGN-U312467	AT1G70730	4.02	glycolysis.PGM
SGN-U326688	AT4G36810	16.1.1.010	secondary metab.isoprenoids.non-mevalonate pathway.
SGN-U314627	AT1G27450	23.3.1.01	nucleotide metab.salvage.phosphoribosyltransferases.aprt
SGN-U314504	AT3G02090	29.3.2	protein.targeting.mitochondria
SGN-U312518	AT1G79550	4.010	glycolysis.phosphoglycerate kinase
SGN-U315827	AT3G15640	9.07	mitochondrial electron transport / ATP synt.cytochrome c oxidase
SGN-U315828	AT3G15640	9.07	mitochondrial electron transport / ATP synt.cytochrome c oxidase
SGN-U340510	ATCG00130	1.1.04	PS.lightreaction.ATP synth.
SGN-U314735	AT5G55480	11.9.3.03	lipid metab.lipid degrad.lysophospholipases.
SGN-U312385	AT1G04410	8.2.09	TCA / org. transformation.other organic acid transformaitons.cyt MDH
SGN-U316825	AT3G01280	34.20	transport.porins
SGN-U319050	AT5G16150	2.2.2.06, 4.2	major CHO metab.degrad.starch.transporter, transport.sugars
SGN-U313480	AT4G04020	31.1	cell.organisation
SGN-U320667	AT3G57050	13.1.3.4.02	aa metab.synt.aspartate family.methionine.cystathionine beta-lyase
SGN-U316421	AT4G29840	13.1.3.2.01	aa metab.synt.aspartate family.threonine.threonine synth.
SGN-U330577	AT1G72810	13.1.3.2.01	aa metab.synt.aspartate family.threonine.threonine synth.
SGN-U318615	AT1G77590	11.1.09	lipid metab.FA synt. and FA elongation.long chain fatty acid CoA ligase
SGN-U314508	AT1G11650	27.3.99	RNA.regulation of transcription.unclassified
SGN-U322210	AT5G41790	30.11.1, 33.99	signalling.light.COP9 signalosome, development.unspecified
SGN-U313628	AT2G45740	35.1	not assigned.no ontology
SGN-U313242	AT4G15530	6.05	gluconeogenese/ glyoxylate cycle.pyruvate dikinase
SGN-U320116	AT4G15530	6.05	gluconeogenese/ glyoxylate cycle.pyruvate dikinase
SGN-U318076	AT1G31160	29.4	protein.postranslational modification
SGN-U312981	AT5G28010	20.2.99	stress.abiotic.unspecified
SGN-U317470	AT1G70890	20.2.99	stress.abiotic.unspecified
SGN-U316893	AT1G06760	28.1.3	DNA.synt./chromatin structure.histone
SGN-U324109	AT5G35360	11.1.01	lipid metab.FA synt. and FA elongation.Acetyl CoA Carboxylation
SGN-U327019	AT5G35360	11.1.01	lipid metab.FA synt. and FA elongation.Acetyl CoA Carboxylation
SGN-U316131	AT1G17650	7.1.03	OPP.oxidative PP.6-phosphogluconate dehydrogenase
SGN-U318009	AT4G33650	26.17	misc.dynamin
SGN-U318010	AT4G33650	26.17	misc.dynamin
SGN-U319773	AT4G33650	26.17	misc.dynamin
SGN-U319197	AT2G02500	16.1.1.03	secondary metab.isoprenoids.non-mevalonate pathway.CMS
SGN-U317438	AT1G06620	21.2	redox.ascorbate and glutathione
SGN-U332234	AT5G26742	27.1.2	RNA.processing.RNA helicase
SGN-U342110	AT5G26742	27.1.2	RNA.processing.RNA helicase

SGN-U328177	AT5G19750	35.1	not assigned.no ontology
SGN-U341640	AT1G22530	34.99	transport.misc
SGN-U319244	AT1G68560	26.03	misc.gluco-, galacto- and mannosidases
SGN-U320011	AT1G68560	26.03	misc.gluco-, galacto- and mannosidases
SGN-U316184	AT3G04870	16.1.4.03	secondary metab.isoprenoids.carotenoids.zeta-carotene desaturase
SGN-U335523	AT3G04870	16.1.4.03	secondary metab.isoprenoids.carotenoids.zeta-carotene desaturase
SGN-U323749	ATCG00120	1.1.04	PS.lightreaction.ATP synth.
SGN-U329173	ATCG00120	1.1.04	PS.lightreaction.ATP synth.
SGN-U342407	ATCG00540	1.1.03	PS.lightreaction.cytochrome b6/f
SGN-U344009	ATCG00490	1.3.01	PS.calvin cyle.rubisco large subunit
SGN-U346314	ATCG00490	1.3.01	PS.calvin cyle.rubisco large subunit
SGN-U339515	ATCG00670	29.5	protein.degrad.
SGN-U319145	AT2G35010	21.01	redox.thioredoxin
SGN-U318990	AT5G65620	29.5	protein.degrad.
SGN-U313308	AT5G61410	1.3.011	PS.calvin cyle.RPE
SGN-U317964	AT1G64970	16.1.3.05	secondary metab.isoprenoids.tocopherol biosynt.
SGN-U316687	AT3G14390	13.1.3.5.05	aa metab.synt.aspartate family.lysine.diaminopimelate decarboxylase
SGN-U316688	AT3G14390	13.1.3.5.05	aa metab.synt.aspartate family.lysine.diaminopimelate decarboxylase
SGN-U317019	AT3G25860	11.1.031	lipid metab.FA synt. and FA elongation.pyruvate DH
SGN-U317348	AT4G35630	13.1.5.1.02	aa metab.synt.serine-glycine-cysteine group.serine.
SGN-U314994	AT5G36700	1.2.01	PS.photorespiration.phosphoglycolate phosphatase
SGN-U317146	AT2G34460	35.1	not assigned.no ontology
SGN-U317158	AT1G09795	13.1.7.01	aa metab.synt.histidine.ATP phosphoribosyl transferase
SGN-U315048	AT5G57870	29.2.3	protein.synt.initiation
SGN-U315051	AT5G57870	29.2.3	protein.synt.initiation
SGN-U319506	AT2G38270	34.21	transport.calcium
SGN-U314843	AT5G07350	27.3.73	RNA.regulation of transcription.Zn-finger(CCHC)
SGN-U339115	AT5G07350	27.3.73	RNA.regulation of transcription.Zn-finger(CCHC)
SGN-U316576	AT3G13930	8.1.01.02	TCA / org. transformation.TCA.pyruvate DH.E2
SGN-U316577	AT3G13930	8.1.01.02	TCA / org. transformation.TCA.pyruvate DH.E2
SGN-U315756	AT5G26710	29.1.017	protein.aa activation.glutamate-tRNA ligase
SGN-U321757	AT3G26070	31.1	cell.organisation
SGN-U324308	AT5G19370	26.23	misc.rhodanese
SGN-U314053	AT4G29010	11.9.4.09	lipid metab.lipid degrad.beta-oxidation.mutifunctional
SGN-U314059	AT4G29010	11.9.4.09	lipid metab.lipid degrad.beta-oxidation.mutifunctional
SGN-U321063	AT5G52810	16.99	secondary metab.unspecified
SGN-U318805	AT2G43560	29.6	protein.folding
SGN-U321846	AT1G54570	35.1	not assigned.no ontology
SGN-U316095	AT2G37970	35.1	not assigned.no ontology
SGN-U324520	AT5G65620	29.5	protein.degrad.
SGN-U315550	AT5G62530	13.2.2.2	aa metab.degrad.glutamate family.proline
SGN-U345264	AT1G06950	29.3.3	protein.targeting.chloroplast
SGN-U317989	AT3G27925	29.5.5	protein.degrad.serine protease
SGN-U317459	AT3G56130	11.1.01	lipid metab.FA synt. and FA elongation.Acetyl CoA Carboxylation

SGN-U319901	AT5G08740	9.2.03	mitochondrial electron transport / ATP synt.NADH-DH.type II.
SGN-U317448	AT4G33680	13.1.3.5.03	aa metab.synt.aspartate family.lysine.
SGN-U331547	AT4G31880	35.2	not assigned.unknown
SGN-U313285	AT5G09650	23.4.99	nucleotide metab.phosphotransfer and pyrophosphatases.misc
SGN-U313286	AT5G09650	23.4.99	nucleotide metab.phosphotransfer and pyrophosphatases.misc
SGN-U327554	AT1G11430	33.99	development.unspecified
SGN-U323001	AT1G50170	19.032	tetrapyrrole synt.sirohydrochlorin ferrochelatase
SGN-U319010	AT2G17840	33.99	development.unspecified
SGN-U319898	AT4G39080	34.1	transport.p- and v-ATPases
SGN-U321248	AT2G40600	35.1	not assigned.no ontology
SGN-U313293	AT1G06620	21.2	redox.ascorbate and glutathione
SGN-U330019	AT3G44620	29.4	protein.postranslational modification
SGN-U315396	AT1G07790	28.1.3	DNA.synt./chromatin structure.histone
SGN-U318345	AT1G07790	28.1.3	DNA.synt./chromatin structure.histone
SGN-U341559	AT1G49760	27.1	RNA.processing
SGN-U327678	AT1G08490	30.1.01	signalling.in sugar and nutrient physiology
SGN-U315567	AT5G08280	19.05	tetrapyrrole synt.porphobilinogen deaminase
SGN-U320984	AT2G19940	35.1	not assigned.no ontology
SGN-U317489	AT3G24360	11.9.4.03, 13.2.6.3	lipid metab.lipid degrad., aa metab.degrad.aromatic aa.tryptophan
SGN-U322396	AT1G04420	3.5	minor CHO metab.others
SGN-U319695	AT5G55610	35.2	not assigned.unknown
SGN-U341818	AT4G00030	31.1	cell.organisation
SGN-U319358	AT2G42610	35.2	not assigned.unknown
SGN-U317587	AT4G13430	8.2.03	TCA / org. transformation.other organic acid transformaitons.aconitase
SGN-U322539	AT1G80270	27.3.67	RNA.regulation of transcription.putative transcription regulator
SGN-U316234	AT2G36690	26.07	misc.oxidases - copper, flavone etc.
SGN-U315957	AT3G61870	35.2	not assigned.unknown
SGN-U320539	AT1G77840	29.2.3	protein.synt.initiation
SGN-U316249	AT4G31180	29.1.012	protein.aa activation.aspartate-tRNA ligase
SGN-U315728	AT4G31870	21.2.2	redox.ascorbate and glutathione.glutathione
SGN-U339001	AT1G07790	28.1.3	DNA.synt./chromatin structure.histone
SGN-U317216	AT4G33030	11.10.03	lipid metab.glycolipid synt.UDP-sulfoquinovose synth.
SGN-U318185	AT2G32730	29.5.11.20	protein.degrad.ubiquitin.proteasom
SGN-U324285	AT2G32730	29.5.11.20	protein.degrad.ubiquitin.proteasom
SGN-U322187	AT3G54400	27.3.99	RNA.regulation of transcription.unclassified
SGN-U330998	AT3G07020	11.8.03	lipid metab.'exotics' (steroids, squalene etc)
SGN-U320907	AT3G14590	30.3	signalling.calcium
SGN-U313804	AT3G19170	29.5.07	protein.degrad.metalloprotease
SGN-U315813	AT2G43710	11.1.015	lipid metab.FA synt. and FA elongation.ACP desaturase
SGN-U322092	AT2G43710	11.1.015	lipid metab.FA synt. and FA elongation.ACP desaturase
SGN-U320703	AT1G14410	27.3.67	RNA.regulation of transcription.putative transcription regulator
SGN-U313420	AT1G11650	27.3.99	RNA.regulation of transcription.unclassified
SGN-U319459	AT1G18060	35.2	not assigned.unknown
SGN-U342143	AT5G64860	2.2.2.04	major CHO metab.degrad.starch.D enzyme

SGN-U326854	AT5G09420	29.3.3	protein.targeting.chloroplast
SGN-U314590	AT1G05010	17.5.1.02	hormone metab.ethylene.synt.-degrad.
SGN-U313244	AT5G17330	13.1.1.1.01	aa metab.synt.central aa metab.GABA.Glutamate decarboxylase

*Proteins marked in grey are novel plastidial proteins